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(54) Title: METHODS FOR ENHANCED ANTIGEN PRESENTATION ON ANTIGEN-PRESENTING CELLS AND COMPOSITIONS PRODUCED THEREBY

(57) **Abstract**

The present invention provides compositions and methods for immunotherapy, and in particular for inducing an immune response against an antigen in a patient. Thus, in one aspect, this invention provides genetically modified antigen-presenting cells which are more potent presenters of exogenous peptide than parental antigen-presenting cells. Compositions comprising these genetically modified cells and a carrier, such as pharmaceutically acceptable carrier, are further provided by this invention. The genetically modified antigen-presenting cells of this invention can be used in adoptive immunotherapy or to expand a substantially pure population of immune effector cells. Methods for expansion of the substantially pure population of cells are also provided by this invention.

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METHODS FOR ENHANCED ANTIGEN PRESENTATION ON ANTIGEN-PRESENTING CELLS AND COMPOSITIONS PRODUCED THEREBY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Application No. 60/078,880, filed March 20, 1998, the contents of which are hereby incorporated by reference into the present disclosure.

TECHNICAL FIELD

This invention is in the field of molecular immunology and medicine. In particular, compositions and methods for improved immunotherapy and cancer vaccines are provided.

BACKGROUND

Immunotherapy of cancer has traditionally been categorized as active (e.g., cancer vaccines), passive (e.g., adoptive cellular therapy or monoclonal antibody therapy), and non-specific (e.g., cytokine therapies). These therapies exploit the discovery that antitumor immune responses occur and can be identified. Genes coding for tumor-associated antigens yielding peptides recognized by antitumor-specific cyotoxic T-lymphocytes (CTLs) have been cloned and characterized. Beyond CTLs, different effector and accessory cells, including NK cells, eosinophils, T helper lymphocytes, macrophages, dendritic cells are believed to cooperate to generate an effective immune response.

Tumor specific T cells, derived from cancer patients, will bind and lyse tumor cells. This specificity is based on their ability to recognize short amino acid sequences (epitopes) presented on the surface of the tumor cells by MHC class I and class II molecules. Insights into the requirements for antigen presentation to T cells have led to the recognition that tumor cells are frequently deficient in

many of the essential components of this process. For example, in general, they lack co-stimulatory molecules, such as B-7 which are required to activate T cells. They often down-regulate their class I MHC molecules (either directly or by failing to express β-2 microglobulin, which is required for surface expression of class I MHC.) Class II molecules are crucial for presenting peptides that elicit $CD4^+$ helper phenotype T cells, which are important for promoting maturation of cytotoxic $CD8^+$ cells. These CTLs destroy the tumor by recognizing a peptide presented by class I molecules.

The availability of specific anti-tumor T cells has enabled the identification of tumor antigens and subsequently the generation of cancer vaccines designed to provoke an anti-tumor immune response. A critical target of vaccines is the specialized antigen-presenting cell ("APC"), the most immunologically powerful of which are the bone marrow- and peripheral blood monocyte-derived dendritic cell ("DC") which can present antigen to T cells in the context of co-stimulatory molecules required for T cell activation. Developing immunization strategies to optimize antigen presentation by dendritic cells is a rational approach to vaccine design.

While recent preclinical studies performed in mouse tumor models are paving the way for clinical trials, a number of considerations remain that will impact significantly whether or not this approach is a viable alternative to standard cancer therapies. One consideration is the appropriate and effective presentation of antigens to CTLs *in vivo*. It has long been known that increased expression of class I MHC proteins on tumor cells enhances their ability to be lysed by tumor specific CTL *in vitro*. Pardoll, "Gene Modified Tumor Vaccines" in Forni et al., CYTOKINE-INDUCED TUMOR IMMUNOGENICITY, Academic Press, San Diego, 1994, pages 71-85. Despite earlier success by enhancing MHC I expression by gene transduction, the relationship between levels of MHC expression and tumorigenicity remains unknown. Futhermore, enhanced expression of self-class I MHC molecules does not always increase the immunologic potency of a tumor

when used in a vaccine capacity, with or without adjuvant. Pardoll, *supra* at page 75.

Therefore, a need exists to enhance antigen presentation by antigen-presenting cells in the context of a cancer vaccine and therapies. This invention satisfies this need and provides related advantages as well.

DISCLOSURE OF THE INVENTION

The present invention provides compositions and methods for immunotherapy, and in particular for inducing an immune response against an antigen in a patient. Thus, in one aspect, this invention provides a genetically modified antigen-presenting cell lacking an effective endogenous transporter associated with antigen processing ("TAP") activity and presenting exogenous antigen on major histocompatibility complex class I molecule. The genetically modified cells of this invention are more potent presenters of exogenous peptide than parental antigen-presenting cells.

Compositions comprising these genetically modified cells and a carrier, such as a pharmaceutically acceptable carrier, are further provided by this invention. The cells and compositions containing these cells are used as active cancer vaccines.

The genetically modified antigen-presenting cells of this invention also can be used to expand a substantially pure population of immune effector cells. Methods for expansion of the substantially pure population of cells are also provided by this invention.

This invention further provides a method for enhancing an immune response against an antigen by administering to a subject an effective amount of an agent that inhibits endogenous TAP activity and an effective amount of the antigen, under conditions which favor the presentation of the antigen on a major histocompatibility complex class I molecule of an antigen-presenting cell.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B (Seq. ID Nos. 1 to 5) depicts the coding sequence for HSV-1 and HSV-2 ICP47.

Figure 2 (Seq. ID Nos.6 to 10) depicts the murine and human amino acid sequences for the melanoma antigen gp100. Minimal essential epitopes are identified in bold lettering. The nucleotide coding sequence for each antigen can be deduced from the amino acid sequence.

Figures 3A and 3B (Seq. ID Nos. 11 to 18) depicts the coding sequence for human and murine melanoma antigen MART1.

Figure 4 (Seq. ID Nos. 19 and 20) depicts the coding sequence and the predicted amino acid sequence for the human antigen MART1. The hydrophobic region is underlined.

Figures 5A and %B (Seq. ID Nos. 21 and 22) are the coding sequences for the mouse and human tyrosinase-related-protein-2 cDNA. CTL epitope is underlined.

Figure 6 is a graph summaring the results of a CTL assay of infected human dendritic cells transduced with ICP47. The figure shows that coexpression of HSV ICP47 with gp100 reduces recognition of the HLA-A2-restricted gp100 epitope, G9-209 by CTL.

Figure 7 is a graph showing HSV ICP47 infected dendritic cells pulsed with the gp100 epitope, G9-209, are more efficient at presenting G9-209 peptide than cells with intact endogenous antigen presentation capacity.

MODES FOR CARRYING OUT THE INVENTION

Throughout this disclosure, various publications, patents and published patent specifications are referenced by an identifying citation. The disclosures of these publications, patents and published patent specifications are hereby

incorporated by reference into the present disclosure to more fully describe the state of the art to which this invention pertains.

Definitions

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of immunology, molecular biology, microbiology, cell biology and recombinant DNA, which are within the skill of the art. See, e.g., Sambrook, Fritsch and Maniatis, MOLECULAR CLONING: A LABORATORY MANUAL, 2nd edition (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel et al. eds., (1987)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.): PCR 2: A PRACTICAL APPROACH (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1989) ANTIBODIES, A LABORATORY MANUAL, and ANIMAL CELL CULTURE (R.I. Freshney, ed. (1987)).

As used herein, certain terms may have the following defined meanings.

As used in the specification and claims, the singular form "a" "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

The term "genetically modified" means containing and/or expressing a foreign gene, polynucleotide or nucleic acid sequence which in turn, modifies the genotype or phenotype of the cell or its progeny. In other words, it refers to any addition, deletion or disruption to a cell's endogenous nucleotides.

"Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogstein binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more

extensive process, such as the initiation of a PCR reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

Examples of stringent hybridization conditions include: incubation temperatures of about 25°C to about 37°C; hybridization buffer concentrations of about 6 X SSC to about 10 X SSC; formamide concentrations of about 0% to about 25%; and wash solutions of about 6 X SSC. Examples of moderate hybridization conditions include: incubation temperatures of about 40°C to about 50°C; buffer concentrations of about 9 X SSC to about 2 X SSC; formamide concentrations of about 30% to about 50%; and wash solutions of about 5 X SSC to about 2 X SSC. Examples of high stringency conditions include: incubation temperatures of about 55°C to about 68°C; buffer concentrations of about 1 X SSC to about 0.1 X SSC; formamide concentrations of about 55% to about 75%; and wash solutions of about 1 X SSC, 0.1 X SSC, or deionized water. In general, hybridization incubation times are from 5 minutes to 24 hours, with 1, 2, or more washing steps, and wash incubation times are about 1, 2, or 15 minutes. SSC is 0.15 M NaCl and 15 mM citrate buffer. It is understood that equivalents of SSC using other buffer systems can be employed.

A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 80%, 85%, 90%, or 95%) of "sequence identity" to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987) Supplement 30, section 7.7.18, Table 7.7.1. Preferably, default parameters are used for alignment. A preferred alignment program is BLAST, using default parameters. In particular, preferred programs are BLASTN and BLASTP, using the following default parameters: Genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50

sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + SwissProtein + SPupdate + PIR. Details of these programs can be found at the following Internet address: http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST.

As used herein, the term "cytokine" refers to any one of the numerous factors that exert a variety of effects on cells, for example, inducing growth or proliferation. Non-limiting examples of cytokines which may be used alone or in combination in the practice of the present invention include, interleukin-2 (IL-2), stem cell factor (SCF), interleukin 3 (IL-3), interleukin 6 (IL-6), interleukin 12 (IL-12), G-CSF, granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin-1 alpha (IL-1α), interleukin 11(IL-11), MIP-1α, leukemia inhibitory factor (LIF), c-kit ligand, thrombopoietin (TPO) and flt3 ligand. The present invention also includes culture conditions in which one or more cytokines is specifically excluded from the medium. Cytokines are commercially available from several vendors such as, for example, Genzyme (Framingham, MA), Genentech (South San Francisco, CA), Amgen (Thousand Oaks, CA), R&D Systems and Immunex (Seattle, WA). It is intended, although not always explicitly stated, that molecules having similar biological activity as wild-type or purified cytokines (e.g., recombinantly produced or biologically equivalent variants thereof) are intended to be used within the spirit and scope of the invention.

The terms "major histocompatibility complex" or "MHC" refers to a complex of genes encoding cell-surface molecules that are required for antigen presentation to T cells and for rapid graft rejection. In humans, the MHC complex is also known as the HLA complex. The proteins encoded by the MHC complex are known as "MHC molecules" and are classified into class I and class II MHC molecules. Class I MHC molecules include membrane heterodimeric proteins made up of an □ chain encoded in the MHC associated noncovalently with □2-microglobulin. Class I MHC molecules are expressed by nearly all nucleated cells

and have been shown to function in antigen presentation to CD8+ T cells. Class I molecules include HLA-A, -B, and -C in humans. Class I molecules generally bind peptides 8-10 amino acids in length. Class II MHC molecules also include membrane heterodimeric proteins consisting of noncovalently associated α and β chains. Class II MHC are known to participate in antigen presentation to $CD4^+$ T cells and, in humans, include HLA-DP, -DQ, and DR. Class II molecules generally bind peptides 12-20 amino acid residues in length. The term "MHC restriction" refers to a characteristic of T cells that permits them to recognize antigen only after it is processed and the resulting antigenic peptides are displayed in association with either a self class I or class II MHC molecule. Methods of identifying and comparing MHC are well known in the art and are described in Allen et al. (1994) Human Imm. **40**:25-32; Santamaria et al. (1993) Human Imm. **37**:39-50; and Hurley et al. (1997) Tissue Antigens **50**:401-415.

The term "antigen presenting cell" as used herein intends any cell which presents on its surface an antigen in association with a major histocompatibility complex molecule, or portion thereof, or, alternatively, one or more non-classical MHC molecules, or a portion thereof. Examples of suitable APCs are discussed in detail below and include, but are not limited to, whole cells such as macrophages, dendritic cells, B cells, hybrid APCs, and foster antigen presenting cells. Methods of making hybrid APCs are described and known in the art. WO 98/46785; and WO 95/16775.

Dendritic cells (DCs) are potent antigen-presenting cells. It has been shown that DCs provide all the signals required for T cell activation and proliferation. These signals can be categorized into two types. The first type, which gives specificity to the immune response, is mediated through interaction between the T-cell receptor/CD3 ("TCR/CD3") complex and an antigenic peptide presented by a major histocompatibility complex ("MHC" defined above) class I or II protein on the surface of APCs. This interaction is necessary, but not sufficient, for T cell activation to occur. In fact, without the second type of

signals, the first type of signals can result in T cell anergy. The second type of signals, called co-stimulatory signals, is neither antigen-specific nor MHC-restricted, and can lead to a full proliferation response of T cells and induction of T cell effector functions in the presence of the first type of signals. As used herein, "dendritic cell" is to include, but not be limited to a pulsed dendritic cell, a foster cell or a dendritic cell hybrid.

"Co-stimulatory molecules" are involved in the interaction between receptor-ligand pairs expressed on the surface of antigen presenting cells and T cells. Research accumulated over the past several years has demonstrated convincingly that resting T cells require at least two signals for induction of cytokine gene expression and proliferation (Schwartz R.H. (1990) Science **248**:1349-1356 and Jenkins M.K. (1992) Immunol. Today **13**:69-73). One signal, the one that confers specificity, can be produced by interaction of the TCR/CD3 complex with an appropriate MHC/peptide complex. The second signal is not antigen specific and is termed the "co-stimulatory" signal. This signal was originally defined as an activity provided by bone-marrow-derived accessory cells such as macrophages and dendritic cells, the so called "professional" APCs. Several molecules have been shown to enhance co-stimulatory activity. These are heat stable antigen (HSA) (Liu Y. et al. (1992) J. Exp. Med. **175**:437-445); chondroitin sulfate-modified MHC invariant chain (Ii-CS) (Naujokas M.F. et al. (1993) Cell **74**:257-268); intracellular adhesion molecule 1 (ICAM-1) (Van Seventer, G.A. (1990) J. Immunol. **144**:4579-4586); and B7-1 and B7-2/B70 (Schwartz R.H. (1992) Cell **71**:1065-1068). One exemplary receptor-ligand pair is the B7 co-stimulatory molecule on the surface of APCs and its counter-receptor CD28 or CTLA-4 on T cells (Freeman et al. (1993) Science **262**:909-911; Young et al. (1992) J. Clin. Invest. **90**:229; and Nabavi et al. (1992) Nature **360**:266-268). Other important co-stimulatory molecules are CD40, CD54, CD80, CD86. As used herein, the term "co-stimulatory molecule" encompasses any single molecule or combination of molecules which, when acting together with a peptide/MHC

complex bound by a TCR on the surface of a T cell, provides a co-stimulatory effect which achieves activation of the T cell that binds the peptide. The term thus encompasses B7, or other co-stimulatory molecule(s) on an antigen-presenting matrix such as an APC, fragments thereof (alone, complexed with another molecule(s), or as part of a fusion protein) which, together with peptide/MHC complex, binds to a cognate ligand and results in activation of the T cell when the TCR on the surface of the T cell specifically binds the peptide. Co-stimulatory molecules are commercially available from a variety of sources, including, for example, Beckman Coulter. It is intended, although not always explicitly stated, that molecules having similar biological activity as wild-type or purified co-stimulatory molecules (e.g., recombinantly produced or muteins thereof) are intended to be used within the spirit and scope of the invention.

As used herein, "solid phase support" or "solid support", used interchangeably, is not limited to a specific type of support. Rather a large number of supports are available and are known to one of ordinary skill in the art. Solid phase supports include silica gels, resins, derivatized plastic films, glass beads, cotton, plastic beads, alumina gels. As used herein, "solid support" also includes synthetic antigen-presenting matrices, cells, and liposomes. A suitable solid phase support may be selected on the basis of desired end use and suitability for various protocols. For example, for peptide synthesis, solid phase support may refer to resins such as polystyrene (e.g., PAM-resin obtained from Bachem Inc., Peninsula Laboratories, etc.), POLYHIPE® resin (obtained from Aminotech, Canada), polyamide resin (obtained from Peninsula Laboratories), polystyrene resin grafted with polyethylene glycol (TentaGel®, Rapp Polymere, Tubingen, Germany) or polydimethylacrylamide resin (obtained from Milligen/Biosearch, California).

The term "modulate an immune response" includes inducing (increasing, eliciting) an immune response; and reducing (suppressing) an immune response.

An immunomodulatory method (or protocol) is one that modulates an immune response in a subject.

As used herein, the term "inducing an immune response in a subject" is a term well understood in the art and intends that an increase of at least about 2-fold, more preferably at least about 5-fold, more preferably at least about 10-fold, more preferably at least about 100-fold, even more preferably at least about 500-fold, even more preferably at least about 1000-fold or more in an immune response to an antigen (or epitope) can be detected (measured), after introducing the antigen (or epitope) into the subject, relative to the immune response (if any) before introduction of the antigen (or epitope) into the subject. An immune response to an antigen (or epitope), includes, but is not limited to, production of an antigen-specific (or epitope-specific) antibody, and production of an immune cell expressing on its surface a molecule which specifically binds to an antigen (or epitope). Methods of determining whether an immune response to a given antigen (or epitope) has been induced are well known in the art. For example, antigen-specific antibody can be detected using any of a variety of immunoassays known in the art, including, but not limited to, ELISA, wherein, for example, binding of an antibody in a sample to an immobilized antigen (or epitope) is detected with a detectably-labeled second antibody (e.g., enzyme-labeled mouse anti-human Ig antibody). Immune effector cells specific for the antigen can be detected any of a variety of assays known to those skilled in the art, including, but not limited to, FACS, or, in the case of CTLs, ^{51}Cr-release assays, or ^{3}H-thymidine uptake assays.

The term "immune effector cells" refers to cells capable of binding an antigen and which mediate an immune response. These cells include, but are not limited to, T cells, B cells, monocytes, macrophages, NK cells and cytotoxic T lymphocytes (CTLs), for example CTL lines, CTL clones, and CTLs from tumor, inflammatory, or other infiltrates. Certain diseased tissues express specific antigens and CTLs specific for these antigens have been identified. For example,

approximately 80% of melanomas express the antigen known as GP-100 which contains several CTL epitopes.

A "naïve" cell is a cell that has never been exposed to an antigen.

The term "culturing" refers to the *in vitro* propagation of cells or organisms on or in media of various kinds. It is understood that the descendants of a cell grown in culture may not be completely identical (either morphologically, genetically, or phenotypically) to the parent cell. By "expanded" is meant any proliferation or division of cells.

A "subject" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets.

As used herein, "expression" refers to the process by which polynucleotides are transcribed into mRNA and translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA, if an appropriate eukaryotic host is selected. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Sambrook et al. (1989) *supra*). Similarly, an eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors can be obtained commercially or assembled by the sequences described in methods well known in the art, for example, the methods described above for constructing vectors in general.

The term "sequence motif" refers to a pattern present in a group of molecules (e.g., amino acids or nucleotides). For instance, in one embodiment, the present invention provides for identification of a sequence motif among

peptides. In this embodiment, a typical pattern may be identified by characteristic amino acid residues, such as hydrophobic, hydrophilic, basic, acidic, and the like.

The term "peptide" is used in its broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, e.g. ester, ether, etc. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics. A peptide of three or more amino acids is commonly called an oligopeptide if the peptide chain is short. If the peptide chain is long, the peptide is commonly called a polypeptide or a protein.

An "isolated" or "purified" population of cells is substantially free of cells and materials with which it is associated in nature. By substantially free or substantially purified is meant at least 50% of the population are immune effector cells, preferably at least 70%, more preferably at least 80%, and even more preferably at least 90% free of non-immune effector cells with which they are associated in nature.

A "composition" is intended to mean a combination of active agent and another compound or composition, inert (for example, a detectable agent or label) or active, such as an adjuvant.

A "pharmaceutical composition" is intended to include the combination of an active agent with a carrier, inert or active, making the composition suitable for diagnostic or therapeutic use *in vitro*, *in vivo* or *ex vivo*.

As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Martin, REMINGTON'S PHARM. SCI., 15th Ed. (Mack Publ. Co., Easton (1975)).

An "effective amount" is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages.

As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

This invention relates to the genetic modification of mature antigen-presenting cells ("APC") such that they are deficient in endogenous presentation of processed antigen in the context of MHC class 1 molecules on the cell surface. Such modified cells are better able to present exogenous minimal essential peptide antigens to corresponding immune effector cells such as cytotoxic T lymphocytes. This is achieved by inhibiting endogenous TAP transporters thereby preventing endogenous antigens from complexing with MHC class 1 molecules in the endoplasmic reticulum. Thus, in one aspect, this invention provides a genetically modified antigen-presenting cell lacking effective endogenous TAP transporter activity and presenting exogenous antigen on major histocompatibility complex class I molecule. The genetically modified cells of this invention are more potent presenters of exogenous peptide than parental antigen-presenting cells.

Indeed, the cells of this invention, because they are deficient in endogenous peptide processing, they will provoke a drastically reduced allo-

response (*e.g.*, in the mouse). Crumpacker et al. (1992) J. Immunol. **148(10)**:3004-3011. Moreover, because the cells are also deficient in the accumulation of MHC class I molecules on the cell defective in antigen processing (Elvin et al. (1993) J. Immunol. Methods **158(2)**:161-171) one may expose the APCs of this invention to an MHC allele of a preselected halotype (*e.g.*, HLA-A2) to have the exogenously added peptides presented in the preferred or preselected halotype. Thus, the modified cells of this invention are useful as universal APCs, *i.e.*, they are more suitable for allogeneic use than prior art APCs because one can load the exogenous peptide in a preselected MHC allele, that in one embodiment matches the subject to which the cells will be administered.

The genetically modified cell of this invention is a modified antigen-presenting cell, which may be, but is not limited to, a genetically modified dendritic cell. Antigens may be presented by any variety of methods, including pulsing and fusion with tumor cells.

As used herein, "TAP" encompasses both TAP1 and TAP2 which are members of the protein superfamily of ATP-binding cassette (ABC) transporters. Neumann et al. (1997) J. Mol. Bio. **272**:484-492. The coding sequence for the TAP proteins are disclosed in Beck et al. (1992) J. Mol. Biol. **228(2)**:433-441 and Genbank under Accession numbers X66401 and S57528. The TAP transporters are characterized by highly conserved Walker A/B motifs and the C-loop signature. (For a review see Higgins (1992) Annu. Rev. Cell. Biol. **8**:67-113). Inhibition of the TAP transporters is achieved by expressing in the cell exogenous a polynucleotide encoding a protein or peptide having herpes simplex virus ICP47 biological activity. Small molecules also may be used to inhibit TAP.

A protein or polypeptide having ICP47 biological activity includes mutants, variants, full length and fragments of the open reading frame of ICP47. Neumann et al. (1997) J. Mol. Biol. **272**:484-92, has identified a fragment of 32 amino acid residues (ICP47 (3-34)) that is the minimal region harboring an activity to inhibit peptide-binding to TAP comparable to the full length protein.

Within this actual domain, various mutants and chimeras of ICP47 derived from HSV-1 and HSV-2 have been used to identify amino acid residues critical for TAP inhibition and are useful within the methods of this invention. Galucha et al. (1997) J. Exp. Med. **185(9)**:1565-72, also reported the chemical synthesis of full length ICP47 and show that its biological activity is indistinguishable from that of recombinant ICP47. The sequence of the ICP47 minimally required for TAP inhibition resided within residues 2-35. In addition to the variants disclosed in Neumann et al., *supra*, variants may include proteins and polypeptides have conservative amino acid substitutions or they may be muteins or chimeras, as described below. The variants, muteins and chimeras may be assayed for biological activity using the TAP inhibition assay described in Neumann et al., *supra*, and reproduced below.

It is known within the state of the art that minor modification to a nucleotide sequence will not affect the function of the molecules encoded thereby. Thus, biologically equivalent polynucleotides of published sequences and the sequences disclosed herein are also useful in the methods described herein and are encompassed within peptides having ICP47 biological activity. These polynucleotides can be identified by hybridization under stringent conditions to the sequences disclosed in the published references or those known in the art. Alternatively, the polynucleotides can be identified as being at least 80%, or more preferably, at least 90% or most preferably, at least 95%, identical to the disclosed sequences using sequence alignment programs and default parameters.

Alternatively, polypeptides coding for the ICP47 peptide, fragments thereof or a variant thereof are transduced into the antigen-presenting cells under conditions which favor inhibition of the TAP transporters. APCs then are loaded with antigen using methods well-known in the art and briefly described below. Compositions comprising any of these genetically modified cells and a carrier, such as a pharmaceutically acceptable carrier, are further provided by this invention.

Any antigen which can complex MHC I molecules can be effectively and more potently presented on the genetically modified cells is within the scope of this invention. Such antigens include, but are not limited to a tumor antigen, e.g., a polypeptide comprising a minimal essential epitope derived from gp100, MAGE1, MART, MUC1 and tyrosinase-related-protein-2 (TRP-2). Boon et al. (1995) Immunol. Today **16**:334-336. The sequences of these antigens are provided in Figures 2 through 5. Using these sequences and recombinant expression techniques summarized below, polypeptides and proteins can be made for presentation on the APC of this invention.

MART1 and gp100 are melanocyte differentiation antigens specifically recognized by HLA-A2 restricted tumor-infiltrating lymphocytes (TILs) derived from patients with melanoma, and appear to be involved in tumor regression (Kawakami et al. (1994) Proc. Natl. Acad. Sci. USA **91**:6458-62 and Kawakami et al. (1994) Proc. Natl. Acad. Sci. USA **91**:3515-9). Recently, the mouse homologue of human MART-1 has been isolated. The full-length open reading frame of the mouse MART1 consists of 342 bp, encoding a protein of 113 amino acid residues with a predicted molecular weight of ~13 kDa. Alignment of human and murine MART1 amino acid sequences showed 68.6% identity.

The murine homologue of gp100 has also been identified. The open reading frame consists of 1,878 bp, predicting a protein of 626 amino acid residues which exhibits 75.5% identity to human gp100.

Peptide epitopes associated with pathogenic organisms also can be utilized in the method of this invention. Non-limiting examples include peptides from the influenza nucleoprotein composed of residues 365-80 (NP365-80), NP50-63, and NP147-58 and peptides from influenza hemagglutinin HA202-21 and HA523-45, defined previously in class I restricted cytotoxicity assays. Perkins et al. (1989) J. Exp. Med. **170**: 279-289. Peptides representing epitopes displayed by the malarial parasite *Plasmodium falciparum* have been described. U.S. Patent No. 5,609,872.

A self tissue antigen recognized in autoimmune disorders, *e.g.*,

acetylcholine receptor (AChR) which is recognized in myasthenia gravis, also is intended to be used in the methods described herein. Another class of self antigens for which antigenic epitopes have been described is human chorionic gonadotropin (hCG) beta subunit. U.S. Patent No. 5,733,553. These epitopes find utility in contraceptive methods.

This list of peptides is exemplary only and is not intended to limit the peptides that can used in the methods of the present invention.

Synthetic antigens and altered antigens also can be used in the methods described herein. Synthetic antigenic peptide epitopes have modified amino acid sequences relative to their natural counterparts. Further encompassed by the term "synthetic antigenic peptide" are multimers (concatemers) of a synthetic antigenic peptide of the invention, optionally including intervening amino acid sequences.

Further provided by the present invention are isolated polypeptides comprising synthetic antigenic peptide amino acid sequences of the invention.

Synthetic antigenic peptide epitopes of the present invention can be designed based on known amino acid sequences of antigenic peptide epitopes.

Presentation of the antigen by the APC and binding by the CTL can be confirmed *in vitro* using the procedure described below. New antigens and novel epitopes can be identified using methods well known in the art and described below. The APCs also can be assayed for reactivity to TILs by cytotoxicity and IFN-γrelease assays. These results may be confirmed in an appropriate animal model and prior to clinical testing.

The cells of this invention can be used therapeutically as cancer vaccines or alternatively, in an animal model to test other therapies. Moreover, blocking TAP activity results in a potent decrease in cell surface MHC class I molecules irrespective of the haplotype due to their inherent instability when no peptide is bound. Therefore, when a minimal essential epitope that binds to a single type of allele (e.g., HLA-A2) is supplied exogenously, only these MHCs become stabilized and populate the cell surface. Since MHC I molecules are the dominant

contributors to tissue rejection, TAP-inhibited, peptide pulsed APCs may be used more effectively in therapeutic allogeneic settings where the donor and the recipient need only share the haplotype(s) of the molecules stabilized by the exogenous peptide epitope(s). Thus, by use of this invention to downregulate MHC alleles and repopulation of the APCs with only patient matched alleles (using allele-specific peptides) one can stimulate potent immune responses without the isolation of autologous APCs on a patient-by patient basis. Rather, one can construct a limited series of universal stealth APCs for use in peptide-pulsing immunotherapy. This represents a substantial decrease in the invasiveness and expenses associated with such therapies.

The genetically modified antigen-presenting cells of this invention also can be used to expand a substantially pure population of immune effector cells by growing the immune effector cells in the presence of the APC under conditions which favor the expansion of the immune effector cells at the expense of the APC. In one embodiment, the effector cell is a cytotoxic T lymphocyte (CTL). The substantially pure population of cells expanded by this method and compositions comprising these cells also are claimed herein.

As provided in more detail below, the genetically modified APCs as described hereinabove, are useful diagnostically to screen for other agents having the ability to inhibit TAP activity or therapeutically to induce an immune response in a subject. An effective amount of the genetically modified cell or a composition comprising this cell is administered to the subject under conditions that favor induction of an immune response. In one embodiment, a stimulatory cytokine such as IL-2 or a co-stimulatory factor such as B.7 is administered to the subject. The timing of the administration may be prior to, concurrently, or subsequent to, administration of APCs. Alternatively, adoptive transfer of the immune response can be acheived by administering an effective amount of the substantially pure population of immune effector cells as described above or a composition containing them under conditions that favor maintenance or

expansion of the effector cells transferred in the subject. In aspect, cytokines such as IL-2 also are administered prior to, concurrently, or subsequent to, administration of the immune effector cells.

This invention further provides a method for enhancing an immune response against an antigen, comprising administering to a subject an effective amount of an agent that inhibits endogenous TAP activity and the antigen, under conditions which favor the complexing of the antigen with a major histocompatibility complex class I molecule of an antigen-presenting cell. In a further embodiment, an effective amount of a co-stimulatory molecule or cytokine or a polynucleotide encoding the same is administered to the subject. Although any agent which inhibits TAP is useful in this method, the inventors have found that a herpes simplex virus (HSV-1 and HSV-2) ICP47 peptide or a variant thereof is suitably used in this method. The ICP47 peptide can be administered as a polynucleotide using methods known in the art and described below.

The following examples are intended to illustrate, but not limit, the invention.

Materials and Methods

Various methods are known for the isolation of APCs. These methods are known in the art but are provided below to more fully describe the state of the art.

Isolation, Culturing and Expansion of APCs, Including Dendritic Cells

In one aspect of the invention, the method described in Romani et al (1996) J. Immunol. Methods **196**:135-151 and Bender et al (1996) J. Immunol. Methods **196**:121-135, are used to generate both immature and mature dendritic cells from the peripheral blood mononuclear cells (PBMC) of a mammal, such as a murine, simian or human. Briefly, isolated PBMC are pre-treated to deplete T- and B-cells by means of an immunomagnetic technique. Lymphocyte-depleted PBMC are then cultured for 7 days in RPMI medium, supplemented with 1%

autologous human plasma and GM-CSF/IL-4, to generate dendritic cells. Dendritic cells are nonadherent when compared to their monocyte progenitors. Thus, on day 7, non-adherent cells are harvested for further processing.

The dendritic cells derived from PBMC in the presence of GM-CSF and IL-4 are immature, in that they can lost the nonadherence property and revert back to macrophage cell fate if the cytokine stimuli are removed from the culture. The dendritic cells in an immature state are very effective in processing native protein antigens for the MHC class II restricted pathway (Romani et al. (1989) J. Exp. Med. **169**:1169.

Further maturation of cultured dendritic cells is accomplished by culturing for 3 days in a macrophage-conditioned medium (CM), which contains the necessary maturation factors. Mature dendritic cells are less able to capture new proteins for presentation but are much better at stimulating resting T cells (both $CD4^+$ and $CD8^+$) to grow and differentiate.

Mature dendritic cells can be identified by their change in morphology, such as the formation of more motile cytoplasmic processes; by their nonadherence; by the presence of at least one of the following markers: CD83, CD68, HLA-DR or CD86; or by the loss of Fc receptors such as CD115 (reviewed in Steinman (1991) Annu. Rev. Immunol. **9**:271).

Mature dendritic cells can be collected and analyzed using typical cytofluorography and cell sorting techniques and devices, such as FACScan and FACStar. Primary antibodies used for flow cytometry are those specific to cell surface antigens of mature dendritic cells and are commercially available. Secondary antibodies can be biotinylated Igs followed by FITC- or PE-conjugated streptavidin.

Alternatively, others have reported that a method for upregulating (activating) dendritic cells and converting monocytes to an activated dendritic cell phenotype. This method involves the addition of calcium ionophore to the culture media convert monocytes into activated dendritic cells. Adding the calcium

ionophore A23187, for example, at the beginning of a 24-48 hour culture period resulted in uniform activation and dendritic cell phenotypic conversion of the pooled "monocyte plus DC" fractions: characteristically, the activated population becomes uniformly CD14 (Leu M3) negative, and upregulates HLA-DR, HLA-DQ, ICAM-1, B7.1, and B7.2. Furthermore this activated bulk population functions as well on a small numbers basis as a further purified.

Specific combination(s) of cytokines have been used successfully to amplify (or partially substitute) for the activation/conversion achieved with calcium ionophore: these cytokines include but are not limited to G-CSF, GM-CSF, IL-2, and IL-4. Each cytokine when given alone is inadequate for optimal upregulation.

The second approach for isolating APCs is to collect the relatively large numbers of precommitted APCs already circulating in the blood. Previous techniques for isolating committed APCs from human peripheral blood have involved combinations of physical procedures such as metrizamide gradients and adherence/nonadherence steps (Freudenthal et al. (1990) PNAS **87**:7698-7702); Percoll gradient separations (Mehta-Damani et al. (1994) J. Immunol. **153**:996-1003); and fluorescence activated cell sorting techniques (Thomas et al. (1993) J. Immunol. **151**:6840-52).

In one embodiment, the APCs and therefore the cells presenting one or more antigens are autologous. In another embodiment, the APCs presenting the antigen are allogeneic, i.e., derived from a different subject.

In one aspect, the agent which inhibits TAP activity in the cell is a gene coding for an HSV ICP47 polypeptide or protein. The following compositions and methods can be used to transduce the gene into isolated APC. These methods can be utilized for the insertion of other agents having the same biological activity as HSV ICP47, e.g., a small molecule.

Compositions and Methods of Making Vectors Expressing HSV ICP47 and Gene Encoding Same

The complete sequence for the gene encoding the ICP47 protein is reported by Rixon and McGeoch (1984) Nucleic Acids Res. **12**:2473-87; McGeoch et al. (1985) J. Mol. Biol. **181**:1-13; Begoña et al. (1997) J. Exp. Med. **185**:1565-72. ICP47 produced in bacteria can block human, but not mouse TAP, and heat denaturation of ICP47 has not been shown to affect its ability to block TAP. Tomazin (1996) EMBO J. **15(13)**:3256-3266. Krass et al. (1997) J. Mol. Biol. **272(4)**:484-492 have reported that a fragment of about 32 amino acid residues ICP47 (3-34) is the minimal region harboring activity to inhibit peptide-binding to TAP comparable to the full length protein and therefore representing the active domain. Moreover, it was reported that neither N- nor C-terminal truncations cause an abrupt loss in biological activity. Critical amino acid residues within mutants and chimeras of ICP47 derived from HSV-1 and HSV-2 helped identify amino acid residues critical for TAP inhibition. The sequences encoding these fragments, mutants and chimeras are transduced into the APC and expressed.

These HSV ICP47 fragments, mutants and chimeras can be used for comparison when screening for other agents such as chemicals or polynucleotides, that have the same or enhanced ability to inhibit TAP. The potential agent is inserted or contacted with the APC prior to antigen presentation. The peptides which are to be complexed with the MHC I are then contacted with the cell under suitable conditions which favor presentation of the peptide or protein on the surface of the cell. The ability of the enhanced cell is then assayed and compared to the control cell which has been transduced with an ICP47 peptide or protein prior to presentation with antigen.

As used herein, a "genetic modification" refers to any addition, deletion or disruption to a cell's normal nucleotides. Any method which can achieve the genetic modification of APCs are within the spirit and scope of this invention. Art

recognized methods include viral mediated gene transfer, liposome mediated transfer, transformation, transfection and transduction, e.g., viral mediated gene transfer such as the use of vectors based on DNA viruses such as adenovirus, adeno-associated virus and herpes virus, as well as retroviral based vectors. Arthur et al. (1997) Cancer Gene Therapy **4(1)**:17-25 reports a comparison of gene transfer methods in human dendritic cells.

A "viral vector" is defined as a recombinantly produced virus or viral particle that comprises a polynucleotide to be delivered into a host cell, either *in vivo*, *ex vivo* or *in vitro*. Examples of viral vectors include retroviral vectors, adenovirus vectors, adeno-associated virus vectors and the like. In aspects where gene transfer is mediated by a retroviral vector, a vector construct refers to the polynucleotide comprising the retroviral genome or part thereof, and a therapeutic gene.

As used herein, "retroviral mediated gene transfer" or "retroviral transduction" carries the same meaning and refers to the process by which a gene or nucleic acid sequences are stably transferred into the host cell by virtue of the virus entering the cell and integrating its genome into the host cell genome. The virus can enter the host cell via its normal mechanism of infection or be modified such that it binds to a different host cell surface receptor or ligand to enter the cell. As used herein, retroviral vector refers to a viral particle capable of introducing exogenous nucleic acid into a cell through a viral or viral-like entry mechanism.

Retroviruses carry their genetic information in the form of RNA; however, once the virus infects a cell, the RNA is reverse-transcribed into the DNA form which integrates into the genomic DNA of the infected cell. The integrated DNA form is called a provirus.

In aspects where gene transfer is mediated by a DNA viral vector, such as a adenovirus (Ad) or adeno-associated virus (AAV), a vector construct refers to the polynucleotide comprising the viral genome or part thereof, and a therapeutic gene. Adenoviruses (Ads) are a relatively well characterized, homogenous group

of viruses, including over 50 serotypes. (see, e.g., WO 95/27071) Ads are easy to grow and do not integrate into the host cell genome. Recombinant Ad-derived vectors, particularly those that reduce the potential for recombination and generation of wild-type virus, have also been constructed. (see, WO 95/00655; WO 95/11984).

Adeno-associated virus (AAV) has also been used as a gene transfer system. (See, e.g., U.S. Patent Nos. 5,693,531 and 5,691,176). Wild-type AAV has high infectivity and specificity in integrating into the host cells genome. (Hermonat and Muzyczka (1984) PNAS USA **81**:6466-6470 and Lebkowski et al. (1988) Mol. Cell. Biol. **8**:3988-3996). Recombinant AAV vectors have been produced in high titers and which can transduce target cells at high efficiency.

Vectors that contain both a promoter and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA *in vitro* or *in vivo*, and are commercially available from sources such as Stratagene (La Jolla, CA) and Promega Biotech (Madison, WI). In order to optimize expression and/or *in vitro* transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. Examples of vectors are viruses, such as baculovirus and retrovirus, bacteriophage, cosmid, plasmid, fungal vectors and other recombination vehicles typically used in the art which have been described for expression in a variety of eukaryotic and prokaryotic hosts, and may be used for gene therapy as well as for simple protein expression.

Among these are several non-viral vectors, including DNA/liposome complexes, and targeted viral protein DNA complexes. To enhance delivery to a cell, the nucleic acid or capsid proteins of this invention can be conjugated to

antibodies or binding fragments thereof which bind cell surface antigens. Liposomes that also comprise a targeting antibody or fragment thereof can be used in the methods of this invention. This invention also provides the targeting complexes for use in the methods disclosed herein.

Polynucleotides are inserted into vector genomes using methods well known in the art. For example, insert and vector DNA can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of restricted polynucleotide. These synthetic linkers contain nucleic acid sequences that correspond to a particular restriction site in the vector DNA. Additionally, an oligonucleotide containing a termination codon and an appropriate restriction site can be ligated for insertion into a vector containing, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; versatile multiple cloning sites; and T7 and SP6 RNA promoters for *in vitro* transcription of sense and antisense RNA. Other means are well known and available in the art.

The APCs may be further modified by transduction with a gene coding for a cytokine, such as IL-2 or a co-stimulatory molecule.

While previously characterized antigens and epitopes are suitably expressed in the method of this invention, presentation of yet unidentified antigens and epitopes can also be enhanced. The following is but one means to identify novel antigenic peptides for use in this invention.

Identification of Novel Antigens and Epitopes

Any conventional method, e.g., subtractive library, comparative Northern and/or Western blot analysis of normal and tumor cells, Serial Analysis of Gene Expression (U.S. Patent No. 5,695,937) and SPHERE (described in PCT WO 97/35035), can be used to identify putative antigens for use in the subject invention.

Expression cloning methodology as described in Kawakami et al. (1994) PNAS **91**:3515-19, also can be used to identify a novel tumor-associated antigen. Briefly, in this method, a library of cDNAs corresponding to mRNAs derived from tumor cells is cloned into an expression vector and introduced into target cells which are subsequently incubated with cytotoxic T cells. One identifies pools of cDNAs that are able to stimulate the CTL and through a process of sequential dilution and re-testing of less complex pools of cDNAs one is able to derive unique cDNA sequences that are able to stimulate the CTL and thus encode the cognate tumor antigen, comparative Northern and/or Western blot analysis of normal and tumor cells.

SAGE analysis can be employed to identify the antigens recognized by expanded immune effector cells such as CTLs, by identifying nucleotide sequences expressed in the antigen-expressing cells. Briefly, SAGE analysis begins with providing complementary deoxyribonucleic acid (cDNA) from (1) the antigen-expressing population and (2) cells not expressing that antigen. Both cDNAs can be linked to primer sites. Sequence tags are then created, for example, using the appropriate primers to amplify the DNA. By measuring the differences in these tag sets between the two cell types, sequences which are aberrantly expressed in the antigen-expressing cell population can be identified.

Another method to identify optimal epitopes and new antigenic peptides is a technique known as Solid **PH**ase **E**pitope **RE**covery ("SPHERE"). This method is described in detail in PCT WO 97/35035. Briefly, SHPERE provides an

empirical screening method for the identification of MHC class I-restricted CTL epitopes by utilizing peptide libraries synthesized on beads where each bead contains a unique peptide that can be released in a controlled manner. Roughly speaking, ten 96-well plates with 1000 beads per well will accommodate 10^6 beads; ten 96-well plates with 100 beads per well will accommodate 10^5 beads. In order to minimize both the number of CTL cells required per screen and the amount of manual manipulations, the eluted peptides can be further pooled to yield wells with any desired complexity. For example, based on experiments with soluble libraries, it should be possible to screen 10^7 peptides in 96-well plates (10,000 peptides per well) with as few as 2 X 10^6 CTL cells. After cleaving a percentage of the peptides from the beads, incubating them with gamma-irradiated foster APCs and the cloned CTL line(s), positive wells determined by ^{3}H-thymidine incorporation will be further examined. Alternatively, as pointed out above, cytokine production or cytolytic ^{51}Cr-release assays may be used (Courie et al. (1992) Int. J. Cancer **50**:289-291). Beads from each positive well will be separated and assayed individually as before, utilizing an additional percentage of the peptide from each bead. Positive individual beads will then be decoded, identifying the reactive-amino acid sequence. Analysis of all positives will give a partial profile of conservatively substituted epitopes which stimulate the CTL clone tested. At this point, the peptide can be resynthesized and retested. Also, a second library (of minimal complexity) can be synthesized with representations of all conservative substitutions in order to enumerate the complete spectrum of derivatives tolerated by a particular CTL. By screening multiple CTLs (of the same MHC restriction) simultaneously, the search for crossreacting epitopes is greatly facilitated.

In vitro confirmation of the immunogenicity of an putative antigen of this invention can be confirmed using the method described below which assays for the induction of CTLs.

After isolation of the epitope or antigen, it can be expressed and purified using methods known in the art.

Production of Epitope or Antigen

Most preferably, isolated peptides of the present invention can be synthesized using an appropriate solid state synthetic procedure. Steward and Young, Solid Phase Peptide Synthesis, Freemantle, San Francisco, Calif. (1968). A preferred method is the Merrifield process. Merrifield, Recent Progress in Hormone Res., **23**:451 (1967). The antigenic activity of these peptides may conveniently be tested using, for example, the assays as described herein.

Once an isolated peptide of the invention is obtained, it may be purified by standard methods including chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for protein purification. For immunoaffinity chromatography, an epitope may be isolated by binding it to an affinity column comprising antibodies that were raised against that peptide, or a related peptide of the invention, and were affixed to a stationary support.

Alternatively, affinity tags such as hexa-His (Invitrogen), Maltose binding domain (New England Biolabs), influenza coat sequence (Kolodziej et al. (1991) Methods Enzymol. **194**:508-509), and glutathione-S-transferasc can be attached to the peptides of the invention to allow easy purification by passage over an appropriate affinity column. Isolated peptides can also be physically characterized using such techniques as proteolysis, nuclear magnetic resonance, and x-ray crystallography.

Also included within the scope of the invention are antigenic peptides that are differentially modified during or after translation, e.g., by phosphorylation, glycosylation, crosslinking, acylation, proteolytic cleavage, linkage to an antibody molecule, membrane molecule or other ligand, (Ferguson et al. (1988) Ann. Rev. Biochem. **57**:285-320).

Another aspect of the invention is isolated nucleic acid sequences that encode the novel antigenic peptides described herein. With regard to nucleic acid sequences of the present invention, "isolated" means: an RNA or DNA polymer, portion of genomic nucleic acid, cDNA, or synthetic nucleic acid which, by virtue of its origin or manipulation: (i) is not associated with all of a nucleic acid with which it is associated in nature (e.g. is present in a host cell as a portion of an expression vector); or (ii) is linked to a nucleic acid or other chemical moiety other than that to which it is linked in nature; or (iii) does not occur in nature. By "isolated" it is further meant a nucleic acid sequence: (i) amplified *in vitro* by, for example, polymerase chain reaction (PCR); (ii) synthesized by, for example, chemical synthesis; (iii) recombinantly produced by cloning; or (iv) purified, as by cleavage and gel separation.

The nucleic acid sequences of the present invention may be characterized, isolated, synthesized and purified using no more than ordinary skill. See Sambrook et al. (1989) *supra.*

After a sufficient concentration of peptide is available, the APC containing and expressing the agent that inhibits TAP activity is pulsed with the peptide. After pulsing, the peptide is presented by the APC on the cell surface.

Presentation of Antigen to APCs

Antigen Pulsing

Pulsing is accomplished *in vitro/ex vivo* by exposing APCs to antigenic protein or peptide(s). The protein or peptide(s) are added to APCs at a concentration of 1-10 μm for approximately 3 hours. Paglia et al. (1996) J. Exp. Med. **183**:317, has shown that APC incubated with whole protein *in vitro* were recognized by MHC class I-restricted CTLs, and that immunization of animals with these APCs led to the development of antigen-specific CTLs *in vivo*.

Protein/peptide antigen can also be delivered to APC *in vivo* and presented by the APC. Antigen is preferably delivered with adjuvant via the intravenous, subcutaneous, intranasal, intramuscular or intraperitoneal route of delivery. Grant E.P. and Rock K.L. (1992) J. Immunol. **148**:13; Norbury, C. C. et al. (1995) Immunity **3**:783; and Reise-Sousa C. and Germain R.N. (1995) J. Exp. Med. **182**:841.

Antigen Painting

Another method which can be used is termed "painting". It has been demonstrated that glycosyl-phosphotidylinositol (GPI)-modified proteins possess the ability to reincorporate themselves back into cell membranes after purification. Hirose et al. (1995) Methods Enzymol. **250**:582; Medof et al. (1984) J. Exp. Med. **160**:1558; Medof (1996) FASEB J. **10**:574; and Huang et al. (1994) Immunity **1**:607, have exploited this property in order to create APCs of specific composition for the presentation of antigen to CTLs. Expression vectors for β2-microglobulin and the HLA-A2.1 allele were first devised. The proteins were expressed in Schneider S2 *Drosophila melanogaster* cells, known to support GPI-modification. After purification, the proteins could be incubated together with a purified antigenic peptide which resulted in a trimolecular complex capable of efficiently inserting itself into the membranes of autologous cells. In essence, these protein mixtures were used to "paint" the APC surface, conferring the ability to stimulate a CTL clone that was specific for the antigenic peptide. Cell coating was shown to occur rapidly and to be protein concentration dependent. This method of generating APCs bypasses the need for gene transfer into the APC and permits control of antigenic peptide densities at the cell surfaces.

Hybrid APCs

WO 98/58541 describes a method to fuse cells expressing an antigen with dendritic cells in a manner that the dendritic cells take up and present the antigens

expressed by the antigen-expressing cells. The DCs are fused with the cells in the presence of a fusing agent (e.g., polyethylene glycol or Sendai virus). After culturing the post fusion cell mixture in a medium (which optionally contains hypoxanthine, aminopterin and thymidine) for a period of time (e.g., 5-12 days), the cultured fused cells are separated from unfused non-DC parental cells based on the different adherence properties of the two cell groups. The unfused parental DCs do not proliferate, and so die off.

After the APC is presenting the antigen, it may be used alone or in combination with other compositions and therapies as a cancer vaccine.

Administration of APC as Cancer Vaccine

Prior to clinical use, it is desirable to administer the APCs into a clinically relevant animal model. With respect to human ICP47, it cannot be tested in a mouse model since human ICP47 does not interact with mouse TAP. Human ICP47 does show activity in non-human primates which, however, do not represent a practical assay system for cancer applications.

Several groups have reported the use of allogeneic human peripheral blood lymphocytes in a severe combined immunodeficiency mouse (Hu-PBL-SCID) or the Hu-PBL-SCID-Beige mouse model (Albert et al. (1997) J. Immunol. **159**:1393-1403; Mosier *et al.* (1988) Nature **335**:256; and Parney et al. (1997) Hum. Gen. Ther. **8**:1073-1085). SCID mice lack mature B and T lymphocytes and can be reconstituted with human PBLs. SCID/Beige mice have deficient NK cell activity in addition to their lack of B and T lymphocytes. These mice can be an appropriate animal model for immunization with antigen-pulsed, genetically modified APCs of this invention to induce a response in adoptively transferred PBLs and evaluate protection against a human tumor cell line.

As provided in more detail below, expanded immune effector cells as described herein also have therapeutic applications. Using the model described

above, human immune effector cells (generated *in vitro* by stimulation of peripheral blood lymphocytes wth antigen-pulsed, genetically modified APCs) can be adoptively transferred into SCID, SCID/Beige mice and tested for their ability to protect against human tumor cells.

In addition to administering the pulsed APC as cancer vaccines, they also may be used for the expansion of immune effector cells, which may be used alone or in combination with the pulsed APC in combination therapy.

Expansion of Immune Effector Cells

The present invention makes use of these APCs to stimulate production of an enriched population of antigen-specific immune effector cells. The antigen-specific immune effector cells are expanded at the expense of the APCs, which die in the culture. The process by which naïve immune effector cells become educated by other cells is described essentially in Coulie (1997) Molec. Med. Today 261-268.

The APCs prepared as described above are mixed with naïve immune effector cells. Preferably, the cells may be cultured in the presence of a cytokine, for example IL-2. Because dendritic cells secrete potent immunostimulatory cytokines, such as IL-12, it may not be necessary to add supplemental cytokines during the first and successive rounds of expansion. In any event, the culture conditions are such that the antigen-specific immune effector cells expand (i.e. proliferate) at a much higher rate than the APCs. Multiple infusions of dendritic cells and optional cytokines can be performed to further expand the population of antigen-specific cells.

In one embodiment, the immune effector cells are T cells and are specific for tumor-specific antigens which are presented by the APCs.

Assaying Antigen-Specificity

In a preferred embodiment, the antigen-specific immune effector cells are CTLs. In other words, the cells are selected for their ability to actively lyse the

cells presenting the specific antigen or antigen specificity. Reactivity of the cells can be measured in various ways, including, but not limited to, tritiated thymidine incorporation (indicative of DNA synthesis). In another embodiment, the tetrazolium salt MTT (3-(4,5-dimethyl-thazol-2-yl)-2,5-diphenyl tetrazolium bromide) may be added (Mossman (1983) J. Immunol. Methods **65**:55-63; Niks and Otto (1990) J. Immunol. Methods **130**:140-151). Succinate dehydrogenase, found in mitochondria of viable cells, converts the MTT to formazan blue. Thus, concentrated blue color would indicate metabolically active cells. Similarly, protein synthesis may be shown by incorporation of ^{35}S-methionine. In still another embodiment, cytotoxicity and cell killing assays, such as the classical chromium release assay, may be employed to evaluate epitope-specific CTL activation. Other suitable assays will be known to those of skill in the art.

Compositions

This invention also provides compositions containing any of the above-mentioned proteins, muteins, polypeptides, nucleic acid molecules, vectors, cells antibodies and fragments thereof, and an acceptable solid or liquid carrier. When the compositions are used pharmaceutically, they are combined with a "pharmaceutically acceptable carrier" for diagnostic and therapeutic use. These compositions also can be used for the preparation of medicaments for the diagnosis and treatment of diseases such as cancer.

Adoptive Immunotherapy and Vaccines

The cells and compositions of this invention are useful as cancer vaccines and in adoptive immunotherapy. The ability of autologous antigen-pulsed dendritic cells to induce a clinically relevant immune response has previously been reported. Hsu et al. (1996) Nature Med. **2(1)**:52-58. Using this clinical study as a guide, it is possible to administer an effective amount of the pulsed dendritic cells to a subject to induce an anti-tumor immune response. After isolation and purification of DCs (day 0) purified pulsed dendritic cells were

administered by subcutaneous injection on days 2, 28 and 56 and then 5 to 6 months later. At day 16, patients received subcutaneous injections with either keyhole limpet hemocyanin or idiotype protein in saline at a site separate from intraveneous injection of the pulsed DCs.

The expanded populations of antigen-specific immune effector cells of the present invention also find use in adoptive immunotherapy regimes and as vaccines.

Adoptive immunotherapy methods involve, in one aspect, administering to a subject a substantially pure population of educated, antigen-specific immune effector cells made by culturing naïve immune effector cells with APCs as described above. Preferably, the APCs are dendritic cells.

In one embodiment, the adoptive immunotherapy methods described herein are autologous. In this case, the APCs are made using parental cells isolated from a single subject. The expanded population also employs T cells isolated from that subject. Finally, the expanded population of antigen-specific cells is administered to the same patient.

In another embodiment, the adoptive immunotherapy methods are allogeneic. Here, cells from two or more patients are used to generate the APCs, and stimulate production of the immune effector cells. For instance, cells from other healthy or diseased subjects can be used to generate antigen-specific cells in instances where it is not possible to obtain autologous T cells and/or dendritic cells from the subject providing the biopsy. The expanded population can be administered to any one of the subjects from whom cells were isolated, or to another subject entirely.

In a further embodiment, APCs or immune effector cells are administered with an effective amount of a stimulatory cytokine, such as IL-2 or a co-stimulatory molecule.

The agents identified herein as effective for their intended purpose can be administered to subjects with tumors or those individuals susceptible to or at risk

of developing a tumor. When the agent is administered to a subject such as a mouse, a rat or a human patient, the agent can be added to a pharmaceutically acceptable carrier and systemically or topically administered to the subject. To determine patients that can be beneficially treated, a tumor regression can be assayed. Therapeutic amounts can be empirically determined and will vary with the pathology being treated, the subject being treated and the efficacy and toxicity of the therapy. When delivered to an animal, the method is useful to further confirm efficacy of the agent.

Administration *in vivo* can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. Suitable dosage formulations and methods of administering the agents can be found below.

The agents and compositions of the present invention can be used in the manufacture of medicaments and for the treatment of humans and other animals by administration in accordance with conventional procedures, such as an active ingredient in pharmaceutical compositions.

More particularly, an agent of the present invention also referred to herein as the active ingredient, may be administered for therapy by any suitable route including oral, rectal, nasal, topical (including transdermal, aerosol, buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous and intradermal) and pulmonary. It will also be appreciated that the preferred route will vary with the condition and age of the recipient, and the disease being treated.

Example

Human HLA-A2$^+$ dendritic cells from a normal donor were prepared from peripheral blood mononuclear cells by treatment for 6 days with GM-CSF and IL-4. The cells were infected with adenoviral constructs (250 MOI for each vector), labeled with ^{51}Cr and 5,000 cells of each were incubated with Hurley R 1000 TIL (specific for the human gp100 epitope, G9-209) at the indicated effector: target ratios for hours. Figure 6 shows the results of this experiment. Note that the adenoviral vector expressing HSV ICP47 also expressed the gene for β-GAL. Figure 6 shows that coexpression of ICP47 with gp100 reduces recognition of the HLA-A2 restricted gp100 epitope, G9-209 by CTL.

The second experiment was performed as stated above except that the infected dendritic cells were pulsed with G9-209 peptide for one hour immediately prior to ^{51}Cr labeling. Figure 7 shows that dendritic cells expressing ICP47 are more efficient at presenting G9-209 peptide than cells with intact endogenous antigen presentation capacity.

It is to be understood that while the invention has been described in conjunction with the above embodiments, that the foregoing description and the following examples are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

CLAIMS

1. A genetically modified antigen-presenting cell expressing an effective amount of a polynucleotide coding for a peptide having herpes simplex virus (HSV) ICP47 biological activity and presenting exogenous antigen on a major histocompatibility complex class I molecule.

2. The genetically modified cell of claim 1, wherein the antigen-presenting cell is a dendritic cell.

3. The genetically modified cell of claim 1, wherein the antigen-presenting cell is a peptide-pulsed cell or a hybrid cell.

4. The genetically modifid cell of claim 1, wherein the exogenous antigen is a wild-type antigen, a native antigen, a viral antigen, a self-antigen, an altered antigen, a synthetic antigen or a tumor associated antigen.

5. The genetically modified cell of claim 4, wherein the antigen comprises the minimal essential epitope of the antigen.

6. The genetically modified cell of claim 1, wherein the cell comprises a polynucleotide that encodes a cytokine and/or a co-stimulatory molecule.

7. A method for genetically modifying an antigen-presenting cell comprising introducing into an antigen-presenting cell an effective amount of a polynucleotide expressing a peptide having the biological activity of an herpes simplex virus (HSV) ICP47 peptide and providing the cell with an effective amount of exogenous antigen under conditions which favor association of the

exogenous antigen with a major histocompatibility complex class I molecule, thereby producing a genetically modified antigen-presenting cell.

8. The method of claim 7, wherein the antigen-presenting cell is a dendritic cell.

9. The method of claim 8, wherein presentation of the antigen comprises a method selected from the group consisting of antigen painting, antigen pulsing and fusing the cell with a tumor cell.

10. The method of claim 7, wherein the exogenous antigen is a wild-type antigen, a native antigen, a viral antigen, a self-antigen, an altered antigen, a synthetic antigen or a tumor associated antigen.

11. The method claim 10, wherein the antigen comprises the minimal essential epitope.

12. The method of claim 7, further comprising genetically modifying the cell by introducing a polynucleotide that encodes a cytokine and/or a co-stimulatory molecule.

13. An substantially pure population of immune effector cells grown in the presence of the cell of claim 1 and at the expense of the cell of claim 1.

14. The population of claim 13, wherein the effector cell is a cytotoxic T lymphocyte (CTL).

15. The substantially pure population of immune effector cells of claim 13, wherein the antigen-presenting cell is a genetically modified dendritic cell.

16. The substantially pure population of immune effector cells of claim 13, wherein the antigen-presenting cell is a peptide-pulsed dendritic cell or a hybrid dendritic cell.

17. A method for producing a substantially pure population of immune effector cells comprising culturing the genetically modified cell of claim 1 in the presence of a population of immune effector cells and under conditions which favor expansion of the immune effector cells at the expense of the genetically modified cell, thereby producing a substantially pure population of antigen-specific immune effector cells.

18. The method of claim 17, wherein the antigen-presenting cell is a genetically modified dendritic cell or a pulsed dendritic cell.

19. The method of claim 17, wherein the immune effector cell is a cytotoxic T lymphocyte (CTL).

20. A method of inducing an immune response in a subject comprising administering to the subject an effective amount of the genetically modified cell of claim 1 under conditions that favor induction of an immune response in the subject.

21. The method of claim 27, further comprising administering an effective amount of a cytokine and/or a co-stimulatory molecule to the subject.

22. The method of claim 21, wherein the molecule is administered by administration of a polynucleotide coding for the cytokine and/or co-stimulatory molecule.

```
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    ||||| |||||||||||| ||| | |||||||  || |||||| |  ||||
  1 ATGTCTTGGGCCCTGAAAACGACGGACATGTTTCTGGATTCTTCGCGGTG  50

 51 TGGGCCCAGGACGTACGCCGACGTACGCGATGAGATCAATAAAAGGGGGC 100
    | | |||||||| | ||| ||  ||| |||||| |||||||||| | |
 51 CACACACCGGACGTATGGCGATGTCTGCGCGGAGATCCATAAAAGGGAAC 100

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    | |||||||||| |||||||||||||| || ||| |||||||||||  ||
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151 CTGCTGCGCTCTCCCGGGCTGCTGCC..........CGAAATCGCCC 187
    ||||||| |  |||| |  |||    |          ||||||| | |
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188 CCAACGCATCCTTGGGTGTGGCACATCGAAGAACCGGCGGGACCGTGACC 237
    || | | || |||||||||  | |||||||  | |||| ||| || | |
201 ACAGCAGACCCGTGGGTGTGCTAGATCGAACGAGCGGCAGGATCGCGTGC 250

238 GACAGTCCCCGTAATCCGGTAACCCGTTGA 267
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251 TGGCCCCTTGA                  261
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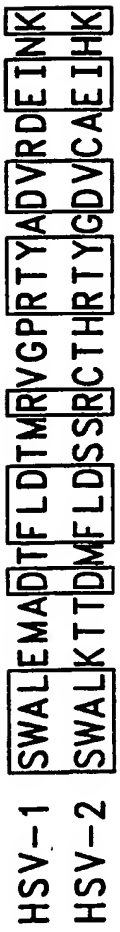
FIG. 1A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HSV-2 RERREDREAARTAVTDPELPLLCPPDVRSDP
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HSV-1 APNASLGVAHRRTGGTVTDSPRNPVTR
HSV-2 ASRNPTQQTRGCARSNERQDRVLAP

FIG. 1B

SUBSTITUTE SHEET (RULE 26)

FIG. 2A

FIG. 2B

} FIG. 2

```
HUMAN     1  MDLVLKRCLL HLAVIGALLA VGATKVPRNQ DWLGVSRQLR TKAWNRQLYP
MOUSE        -*G-QR-SF- PLV-LSA--- ---LEGS--- -----P---V --T-------

          51  EWTEAQRLDC WRGGQVSLKV SNDGPTLIGA NASFSIALNF PGSQKVLPDG
              ----V-GSN- --------R- I------I-- --------H- ----------

         101  QVIWVNNTII NGSQVWGGQP VYPQETDDAC IFPDGGPCPS GSWSQKRSFV
              ----A----- ---------- -----P---- V--------- -PKPP-----

         151  YVWKTWGQYW QVLGGPVSGL SIGTGRAMLG THTMEVTVYH RRGSRSYVPL
              -------K-- --------R- --A--H-K-- ---------- ----Q-----

         201  AHSSSAFTIT DQVPFSVSVS QLRALDGGNK HFLRNQPLTF ALQLHDPSGY
              --A--T---- ---------- --Q----ET- -----H--I- ----------

         251  LAEADLSYTW DFGDSSGTLI SRALVVTHTY LEPGPVTAQV VLQAAIPLTS
              ---------- ----GT---- ----D----- --S-S----- --------V-
```

FIG. 2A

```
301  CGSSPVPGTT DGHRPTAEAP NTTAGQVPTT EVVGTTPGQA PTAEPSGTTS
     ---------- --YM------ G--SR-GT-- ---------M --TQ-----V

351  VQVPTTEVIS TAPVQMPTAE STGMTPEKVP VSEVMGTTLA EMSTPEATGM
     --M-----TA -TSE--L-** ********** *-A-ID---- -V--T-G--T

401  TPAEVSIVVL SGTTAAQVTT TEWVETTARE LPIPEPEGPD ASSIMSTESI
     --T*******P ----V--A-- --********* *********-- --PLLP-Q-S

451  TGGLGPLLDG TATLRLVKRQ VPLDCVLYRY GSFSVTLDIV QGIESAEILQ
     ---IS----D -D-IM----- ---------- ----LA---- ----------

501  AVPSGEGDAF ELTVSCQGGL PKEACMEISS PGCQPPAQRL CQPVLPSPAC
     ---FS----- ---------- ------D--- ---------- --S-P---D-

551  QLVLHQILKG GSGTYCLNVS LADTNSLAVV STQLIMPGQE AGLGQVPLIV
     ------V--- ---------- ---A-----A ----VV---D G----A--L-

601  GILLVLMAVV LASLIYRRRL MKQDFSVPQL PHSSSHWLRL PRIFCSCPIG
     ------V--- -----H-H-- K--G*--S-M --G-T----- -PV-RARGL-

651  ENSPLLSGQQ VX
     ---------- --
```

FIG. 2B

SUBSTITUTE SHEET (RULE 26)

FIG. 3A

FIG. 3A
FIG. 3B
} FIG. 3

A

```
                10        20                30        40        50
mMART   ATGCCCCAAGAAGACATTCACTT------TGGTTATCCCAGGAAGGGGCACAGACGCTCC
        |||||   ||||||   ||||||       |||||| |||| |||||||||| | | |||
hMART   ATGCCAAGAGAAGATGCTCACTTCATCTATGGTTACCCCAAGAAGGGGCACGGCCACTCT
                10        20        30        40        50        60

            60        70        80        90        100       110
mMART   TATGTCACTGCTGAAGAGGCCGCAGGGATCGGCATCCTGATCGTGGTCCTGGGGATTGCT
        ||   |||||||||||||||||| |||||||||||||||||   |||  |||||||  |
hMART   TACACCACGGCTGAAGAGGCCGCTGGGATCGGCATCCTGACAGTGATCCTGGGAGTCTTA
                70        80        90        100       110       120

            120       130       140       150       160       170
mMART   CTGCTTATCGGCTGCTGGTACTGTAGAAGACGAAGTGGATACAGAACCTTGATGGACAAA
        |||||||||||| ||||| |||||||||||||| |||||||||| |||||||||| |||
hMART   CTGCTCATCGGCTGTTGGTATTGTAGAAGACGAAATGGATACAGAGCCTTGATGGATAAA
                130       140       150       160       170       180

            180       190       200       210       220       230
mMART   AGGCGTCATATTGGTATTCAAAAAACCTCAAGGGAAAGATGCTCATGTGAGAGCCCTGAT
        ||  |  ||||  ||||  |  ||||     |||  ||     |||||||  ||    ||   |    ||||
hMART   AGTCTTCATGTTGGCACTCAATGTGCCTTAACAAGAAGATGCCCACAAGAAGGGTTTGAT
                190       200       210       220       230       240
```

```
          240        250        260        270        280        290
mMART     CACCAGGACAGCCGACTGTCTTCTCAAGAGAAATCCCATCAGCCCGTGGTTCCCAACGCT
          || | ||||||||  | |||||  ||||||||||  |  | | || ||||||||||||| |||
hMART     CATCGGGACAGCAAAGTGTCTCTTCAAGAGAAAAACTGTGAACCTGTGGTTCCCAATGCT
                250        260        270        280        290        300

          300        310                  320        330        340
mMART     CCGCCTGCCTATGAGAAGCTCTCT---------TCACCGCCACCTTATTCACCCTGA
          || ||||| |||||||| ||||||         ||||| ||||||||||||||| | |
hMART     CCACCTGCTTATGAGAAACTCTCTGCAGAACAGTCACCACCACCTTATTCACCTTAA
                310        320        330        340        350
```

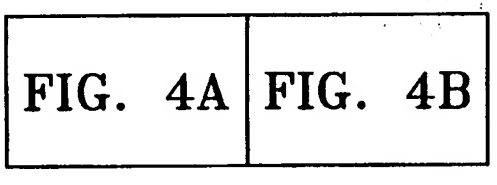
B

```
                    10         20         30         40         50
mMART     MPQEDIHF--GYPRKGHRRSYVTAEEAAGIGILIVVLGIALLIGCWYCRRRSGYRTLMDK
          ||  ||  ||  |||  |||  ||  ||||||||||| | ||  |||||||||||  ||| ||||
hMART     MPREDAHFIYGYPKKGHGHSYTTAEEAAGIGILTVILGVLLLIGCWYCRRRNGYRALMDK
                    10         20         30         40         50         60

          60         70         80         90         100            110
mMART     RRHIGIQKTSRERCSCESPDHQDSRLSSQEKSHQPVVPNAPPAYEKLS---SPPPYSPX
            | | |      ||  |  ||  ||   | |||   ||||||||||||||    |||||||||
hMART     SLHVGTQCALTRRCPQEGFDHRDSKVSLQEKNCEPVVPNAPPAYEKLSAEQSPPPYSPX
                    70         80         90         100        110
```

FIG. 3B

SUBSTITUTE SHEET (RULE 26)

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   1   AGCAGACAGAGGACTCTCATTAAGGAAGG TGTCCTGTGCCTTGACCCTACAAGATGCCA
                                                              MetPro

 120   ACGGCTGAAGAGGCCGCTGGGATCGGCATC CTGACAGTGATCCTGGGAGTCTTACTGCTC
  23   ThrAlaGluGluAlaAlaGlyIleGlyIle LeuThrValIleLeuGlyValLeuLeuLeu

 240   CATGTTGGCACTCAATGTGCCTTAACAAGA AGATGCCCACAAGAAGGGTTTGATCATCGG
  63   HisValGlyThrGlnCysAlaLeuThrArg ArgCysProGlnGluGlyPheAspHisArg

 360   GCTTATGAGAAACTCTCTGCAGAACAGTCA CCACCACCTTATTCACCTTAAGAGCCAGCG
 103   AlaTyrGluLysLeuSerAlaGluGlnSer ProProProTyrSerPro

 480   ATCTAATGTTCTCCTTTGGAATGGTGTAGG AAAAATGCAAGCCATCTCTAATAATAAGTC
 600   TATTAAATTGGGAAAACTCCATCAATAAAT GTTGCAATGCATGATACTATCTGTGCCAGA
 720   GGGGCCATCCAATTTCTCTTTACTTGAAAT TTGGCTAATAACAAACTAGTCAGGTTTTCG
 840   GATACTTTTACAGGTTAAGACAAAGGGTTG ACTGGCCTATTTATCTGATCAAGAACATGT
 960   CTATAGCTCTTTTTTTTTGAGATGGAGTTT CGCTTTTGTTGCCCAGGCTGGAGTGCAATG
1080   CCTCCTGAGTAGCTGGGATTACAGGCGTGC GCCACTATGCCTGACTAATTTTGTAGTTTT
1200   TCTGCCCGCCTCAGCCTCCCAAAGTGCTGG AATTACAGGCGTGAGCCACCACGCCTGGCT
1320   AATGCTATTCTAACTAATGACAAGTATTTT CTACTAAACCAGAAATTGGTAGAAGGATTT
1440   TACCTATGGCAATTTAGCTCTCTTGGGTTC CCAAATCCCTCTCACAAGAATGTGCAGAAG
```

FIG. 4A

```
AGAGAAGATGCTCACTTCATCTATGGTTAC CCCAAGAAGGGGCACGGCCACTCTTACACC 119
ArgGluAspAlaHisPheIleTyrGlyTyr ProLysLysGlyHisGlyHisSerTyrThr  22

ATCGGCTGTTGGTATTGTAGAAGACGAAAT GGATACAGAGCCTTGATGGATAAAAGTCTT 239
IleGlyCysTrpTyrCysArgArgArgAsn GlyTyrArgAlaLeuMetAspLysSerLeu  62

GACAGCAAAGTGTCTCTTCAAGAGAAAAAC TGTGAACCTGTGGTTCCCAATGCTCCACCT 359
AspSerLysValSerLeuGlnGluLysAsn CysGluProValValProAsnAlaProPro 102

AGACACCTGAGACATGCTGAAATTATTTCT CTCACACTTTTGCTTGAATTTAATACAGAC 479
                                                               118

AGTGTTAAAATTTTAGTAGGTCCGCTAGCA GTACTAATCATGTGAGGAAATGATGAGAAA 599
GGTAATGTTAGTAAATCCATGGTGTTATTT TCTGAGAGACAGAATTCAAGTGGGTATTCT 719
AACCTTGACCGACATGAACTGTACACAGAA TTGTTCCAGTACTATGGAGTGCTCACAAAG 839
CAGCAATGTCTCTTTGTGCTCTAAAATTCT ATTATACTACAATAATATTGTAAAGATC 959
GCGCGATCTTGGCTCACCATAACCTCCGCC TCCCAGGTTCAAGCAATTCTCCTGCCTTAG 1079
AGTAGAGACGGGGGTTTCTCCATGTTGGTCA GGCTGGTCTCAAACTCCTGACCTCAGGTGA 1199
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AAATAAGTAAAAGCTACTATGTACTGCCTT AGTGCTGATGCCTGTGTACTGCCTTAAATG 1439
AAATCATAAAGGATCAGAGATTCTGAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAA 1559
```

FIG. 4B

MOUSE TRP2

```
1    MGLVGWGLLL GCLGCGILLR ARAQFPRVCM TLDGVLNKEC CPPLGPEATN
51   ICGFLEGRGQ CAEVQTDTRP WSGPYILRNQ DDREQWPRKF FNRTCKCTGN
101  FAGYNCGGCK FGWTGPDCNR KKPAILRRNI HSLTAQEREQ FLGALDLAKK
151  SIHPDYVITT QHWLGLLGPN GTQPQIANCS VYDFFVWLHY YSVRDTLLGP
201  GRPYKAIDFS HQGPAFVTWH RYHLLWLERE LQRLTGNESF ALPYWNFATG
251  KNECDVCTDD WLGAARQDDP TLISRNSRFS TWEIVCDSLD DYNRRVTLCN
301  GTYEGLLRRN KVGRNNEKLP TLKNVQDCLS LQKFDSPPFF QNSTFSFRNA
351  LEGFDKADGT LDSQVMNLHN LAHSFLNGTN ALPHSAANDP VFVVLHSFTD
401  AIFDEWLKRN NPSTDAWPQE LAPIGHNRMY NMVPFFPPVT NEELFLTAEQ
451  LGYNYAVDLS EEEAPVWSTT LGVVIGILGA FVLLLGLLAF LQYRRLRKGY
501  APLMETGLSS KRYTEEA
```

FIG. 5A

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HUMAN TRP2

```
1    MSPLWWGFLL SCLGCKILPG AQGQFPRVCM TVDSLVNKEC CPRLGAESAN
51   VCGSQQGRGQ CTEVRADTRP WSGPYILRNQ DDRELWPRKF FHRTCKCTGN
101  FAGYNCGDCK FGWTGPNCER KKPPVIRQNI HSLSPQEREQ FLGALDLAKK
151  RVHPDYVITT QHWLGLLGPN GTQPQFANCS VYDFFVWLHY YSVRDTLLGP
201  GRPYRAIDFS HQGPAFVTWH RYHLLCLERD LQRLIGNESF ALPYWNFATG
251  RNECDVCTDQ LFGAARPDDP TLISRNSRFS SWETVCDSLD DYNHLVTLCN
301  GTYEGLLRRN QMGRNSMKLP TLKDIRDCLS LQKFDNPPFF QNSTFSFRNA
351  LEGFDKADGT LDSQVMSLHN LVHSFLNGTN ALPHSAANDP IFVVLHSFTD
401  AIFDEWMKRF NPPADAWPQE LAPIGHNRMY NMVPFFPPVT NEELFLTSDQ
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501  GYTPLMETHL SSKRYTEEA
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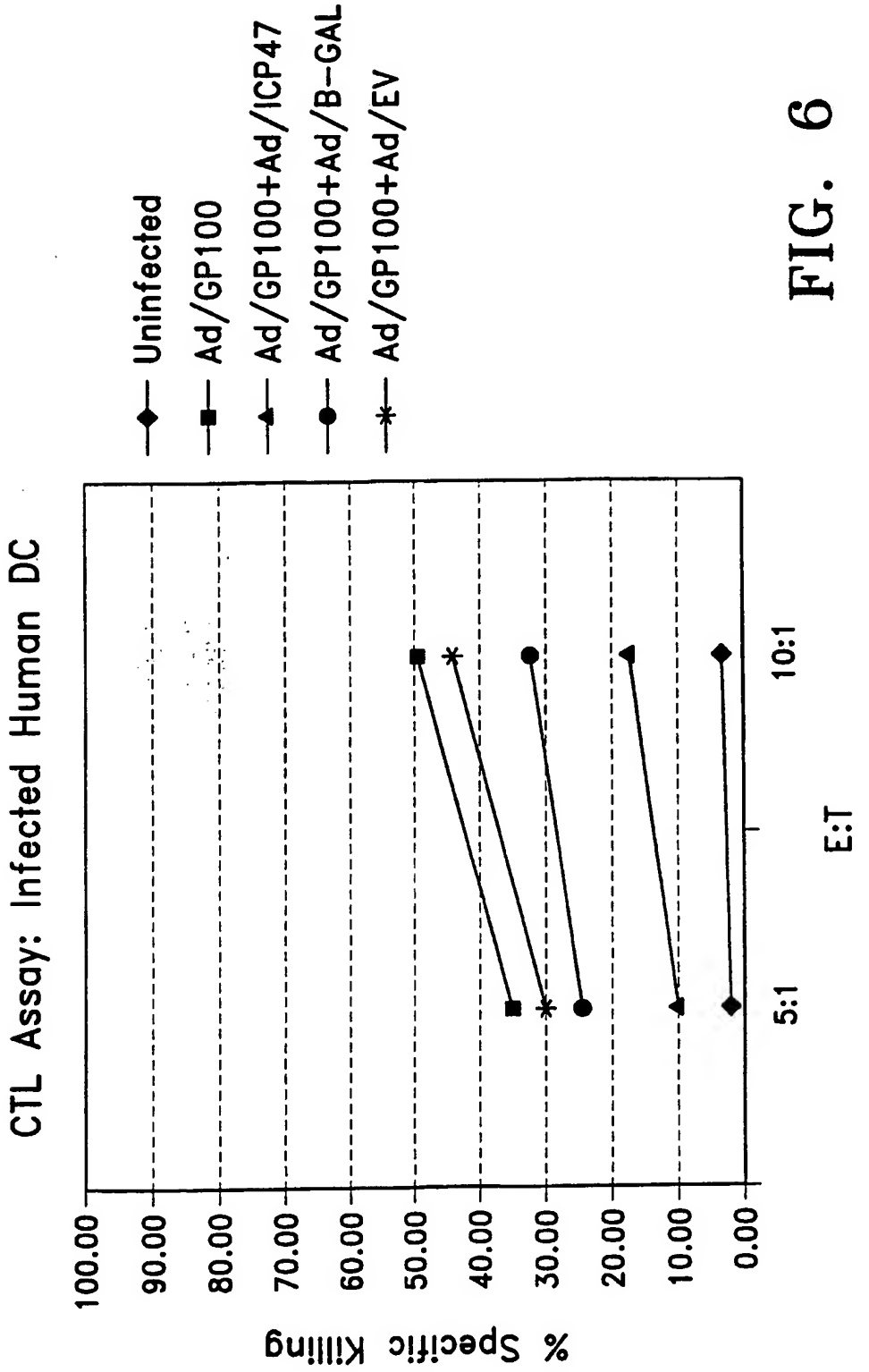
FIG. 5B


CTL Assay: Infected Human DC
Uninfected
Ad/GP100
Ad/GP100+Ad/ICP47
Ad/GP100+Ad/B-GAL
Ad/GP100+Ad/EV
% Specific Killing
100.00
90.00
80.00
70.00
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5:1
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E:T

FIG. 6

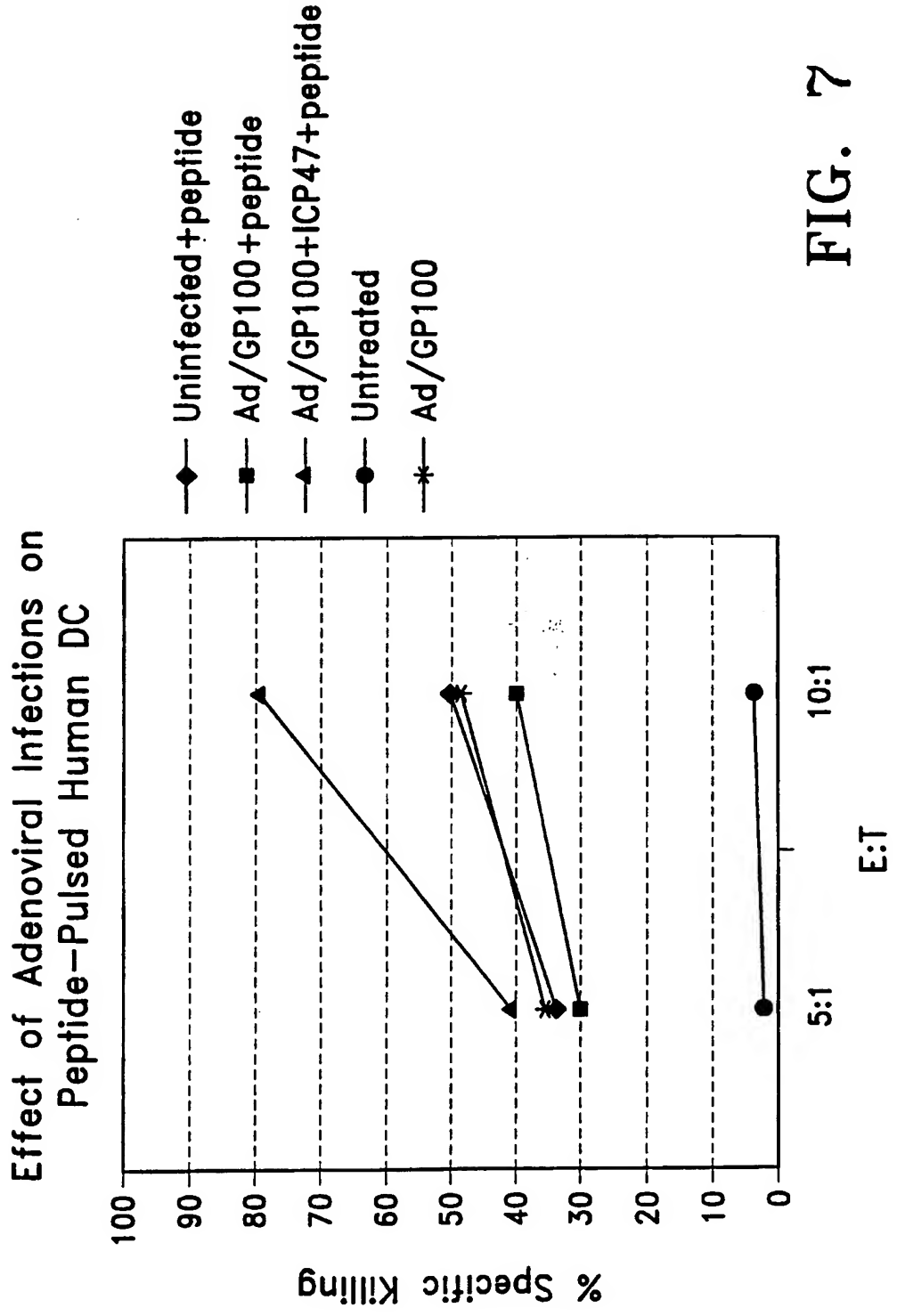
Effect of Adenoviral Infections on Peptide-Pulsed Human DC
Uninfected+peptide
Ad/GP100+peptide
Ad/GP100+ICP47+peptide
Untreated
Ad/GP100
% Specific Killing
100
90
80
70
60
50
40
30
20
10
0
5:1
10:1
E:T

FIG. 7

SEQUENCE LISTING

<110> Nicolette, Charles A.
Kaplan, Johanne
Genzyme Corporation

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Gly Pro Arg Thr Tyr Ala Asp Val Arg Asp Glu Ile Asn Lys Arg Gly
20 25 30

Arg Glu Asp Arg Glu Ala Ala Arg Thr Ala Val His Asp Pro Glu Arg
35 40 45

Pro Leu Leu Arg Ser Pro Gly Leu Leu Pro Glu Ile Ala Pro Asn Ala
50 55 60

Ser Leu Gly Val Ala His Arg Arg Thr Gly Gly Thr Val Thr Asp Ser
65 70 75 80

Pro Arg Asn Pro Val Thr Arg
85

<210> 5
<211> 85
<212> PRT
<213> herpes simplex virus 7

<400> 5
Ser Trp Ala Leu Lys Thr Thr Asp Met Phe Leu Asp Ser Ser Arg Cys
1 5 10 15

Thr His Arg Thr Tyr Gly Asp Val Cys Ala Glu Ile His Lys Arg Glu
20 25 30

Arg Glu Asp Arg Glu Ala Ala Arg Thr Ala Val Thr Asp Pro Glu Leu
35 40 45

Pro Leu Leu Cys Pro Pro Asp Val Arg Ser Asp Pro Ala Ser Arg Asn
50 55 60

Pro Thr Gln Gln Thr Arg Gly Cys Ala Arg Ser Asn Glu Arg Gln Asp
65 70 75 80

Arg Val Leu Ala Pro
85

<210> 6
<211> 662
<212> PRT
<213> Homo sapiens

<400> 6
Met Asp Leu Val Leu Lys Arg Cys Leu Leu His Leu Ala Val Ile Gly
1 5 10 15

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Ala Leu Leu Ala Val Gly Ala Thr Lys Val Pro Arg Asn Gln Asp Trp
            20                  25                  30

Leu Gly Val Ser Arg Gln Leu Arg Thr Lys Ala Trp Asn Arg Gln Leu
        35                  40                  45

Tyr Pro Glu Trp Thr Glu Ala Gln Arg Leu Asp Cys Trp Arg Gly Gly
    50                  55                  60

Gln Val Ser Leu Lys Val Ser Asn Asp Gly Pro Thr Leu Ile Gly Ala
65                  70                  75                  80

Asn Ala Ser Phe Ser Ile Ala Leu Asn Phe Pro Gly Ser Gln Lys Val
                85                  90                  95

Leu Pro Asp Gly Gln Val Ile Trp Val Asn Asn Thr Ile Ile Asn Gly
            100                 105                 110

Ser Gln Val Trp Gly Gly Gln Pro Val Tyr Pro Gln Glu Thr Asp Asp
        115                 120                 125

Ala Cys Ile Phe Pro Asp Gly Gly Pro Cys Pro Ser Gly Ser Trp Ser
    130                 135                 140

Gln Lys Arg Ser Phe Val Tyr Val Trp Lys Thr Trp Gly Gln Tyr Trp
145                 150                 155                 160

Gln Val Leu Gly Gly Pro Val Ser Gly Leu Ser Ile Gly Thr Gly Arg
                165                 170                 175

Ala Met Leu Gly Thr His Thr Met Glu Val Thr Val Tyr His Arg Arg
            180                 185                 190

Gly Ser Arg Ser Tyr Val Pro Leu Ala His Ser Ser Ser Ala Phe Thr
        195                 200                 205

Ile Thr Asp Gln Val Pro Phe Ser Val Ser Val Ser Gln Leu Arg Ala
    210                 215                 220

Leu Asp Gly Gly Asn Lys His Phe Leu Arg Asn Gln Pro Leu Thr Phe
225                 230                 235                 240

Ala Leu Gln Leu His Asp Pro Ser Gly Tyr Leu Ala Glu Ala Asp Leu
                245                 250                 255

Ser Tyr Thr Trp Asp Phe Gly Asp Ser Ser Gly Thr Leu Ile Ser Arg
            260                 265                 270

Ala Leu Val Val Thr His Thr Tyr Leu Glu Pro Gly Pro Val Thr Ala
        275                 280                 285

Gln Val Val Leu Gln Ala Ala Ile Pro Leu Thr Ser Cys Gly Ser Ser
    290                 295                 300

Pro Val Pro Gly Thr Thr Asp Gly His Arg Pro Thr Ala Glu Ala Pro
305                 310                 315                 320

Asn Thr Thr Ala Gly Gln Val Pro Thr Thr Glu Val Val Gly Thr Thr
                325                 330                 335

Pro Gly Gln Ala Pro Thr Ala Glu Pro Ser Gly Thr Thr Ser Val Gln
```

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            340                 345                 350

Val Pro Thr Thr Glu Val Ile Ser Thr Ala Pro Val Gln Met Pro Thr
        355                 360                 365

Ala Glu Ser Thr Gly Met Thr Pro Glu Lys Val Pro Val Ser Glu Val
    370                 375                 380

Met Gly Thr Thr Leu Ala Glu Met Ser Thr Pro Glu Ala Thr Gly Met
385                 390                 395                 400

Thr Pro Ala Glu Val Ser Ile Val Val Leu Ser Gly Thr Thr Ala Ala
                405                 410                 415

Gln Val Thr Thr Thr Glu Trp Val Glu Thr Thr Ala Arg Glu Leu Pro
            420                 425                 430

Ile Pro Glu Pro Glu Gly Pro Asp Ala Ser Ser Ile Met Ser Thr Glu
        435                 440                 445

Ser Ile Thr Gly Ser Leu Gly Pro Leu Leu Asp Gly Thr Ala Thr Leu
    450                 455                 460

Arg Leu Val Lys Arg Gln Val Pro Leu Asp Cys Val Leu Tyr Arg Tyr
465                 470                 475                 480

Gly Ser Phe Ser Val Thr Leu Asp Ile Val Gln Gly Ile Glu Ser Ala
                485                 490                 495

Glu Ile Leu Gln Ala Val Pro Ser Gly Glu Gly Asp Ala Phe Glu Leu
            500                 505                 510

Thr Val Ser Cys Gln Gly Gly Leu Pro Lys Glu Ala Cys Met Glu Ile
        515                 520                 525

Ser Ser Pro Gly Cys Gln Pro Pro Ala Gln Arg Leu Cys Gln Pro Val
    530                 535                 540

Leu Pro Ser Pro Ala Cys Gln Leu Val Leu His Gln Ile Leu Lys Gly
545                 550                 555                 560

Gly Ser Gly Thr Tyr Cys Leu Asn Val Ser Leu Ala Asp Thr Asn Ser
                565                 570                 575

Leu Ala Val Val Ser Thr Gln Leu Ile Met Pro Gly Gln Glu Ala Gly
            580                 585                 590

Leu Gly Gln Val Pro Leu Ile Val Gly Ile Leu Leu Val Leu Met Ala
        595                 600                 605

Val Val Leu Ala Ser Leu Ile Tyr Arg Arg Arg Leu Met Lys Gln Asp
    610                 615                 620

Phe Ser Val Pro Gln Leu Pro His Ser Ser Ser His Trp Leu Arg Leu
625                 630                 635                 640

Pro Arg Ile Phe Cys Ser Cys Pro Ile Gly Glu Asn Ser Pro Leu Leu
                645                 650                 655

Ser Gly Gln Gln Val Xaa
            660
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<210> 7
<211> 4
<212> PRT
<213> Mus musculus

<400> 7
Leu Glu Gly Ser
1

<210> 8
<211> 4
<212> PRT
<213> Mus musculus

<400> 8
Pro Lys Pro Pro
1

<210> 9
<211> 4
<212> PRT
<213> Mus musculus

<400> 9
Pro Leu Leu Pro
1

<210> 10
<211> 5
<212> PRT
<213> Mus musculus

<400> 10
Arg Ala Arg Gly Leu
1 5

<210> 11
<211> 23
<212> DNA
<213> Mus musculus

<400> 11
atgccccaag aagacattca ctt 23

<210> 12
<211> 296
<212> DNA
<213> Mus musculus

<400> 12
tggttatccc aggaaggggc acagacgctc ctatgtcact gctgaagagg ccgcagggat 60

cggcatcctg atcgtggtcc tggggattgc tctgcttatc ggctgctggt actgtagaag 120

acgaagtgga tacagaacct tgatggacaa acaggcgtca tattggtatt caaaaaacct 180

caagggaaag atgctcatgt gagagccctg atcaccagga cagccgactg tcttctcaag 240

agaaatccca tcagcccgtg gttcccaacg ctccgcctgc ctatgagaag ctctct 296

<210> 13
<211> 24
<212> DNA
<213> Mus musculus

<400> 13
tcaccgccac cttattcacc ctga 24

<210> 14
<211> 357
<212> DNA
<213> Homo sapiens

<400> 14
atgccaagag aagatgctca cttcatctat ggttacccca agaaggggca cggccactct 60

tacaccacgg ctgaagaggc cgctgggatc ggcatcctga cagtgatcct gggagtctta 120

ctgctcatcg gctgttggta ttgtagaaga cgaaatggat acagagcctt gatggataaa 180

agtcttcatg ttggcactca atgtgcctta acaagaagat gcccacaaga agggtttgat 240

catcgggaca gcaaagtgtc tcttcaagag aaaaactgtg aacctgtggt tcccaatgct 300

ccacctgctt atgagaaact ctctgcagaa cagtcaccac caccttattc accttaa 357

<210> 15
<211> 8
<212> PRT
<213> Mus musculus

<400> 15
Met Pro Gln Glu Asp Ile His Phe
1 5

<210> 16
<211> 98
<212> PRT
<213> Mus musculus

<400> 16
Gly Tyr Pro Arg Lys Gly His Arg Arg Ser Tyr Val Thr Ala Glu Glu
1 5 10 15

Ala Ala Gly Ile Gly Ile Leu Ile Val Val Leu Gly Ile Ala Leu Leu
20 25 30

Ile Gly Cys Trp Tyr Cys Arg Arg Arg Ser Gly Tyr Arg Thr Leu Met
35 40 45

Asp Lys Arg Arg His Ile Gly Ile Gln Lys Thr Ser Arg Glu Arg Cys

50 55 60

Ser Cys Glu Ser Pro Asp His Gln Asp Ser Arg Leu Ser Ser Gln Glu
65 70 75 80

Lys Ser His Gln Pro Val Val Pro Asn Ala Pro Pro Ala Tyr Glu Lys
85 90 95

Leu Ser

<210> 17
<211> 8
<212> PRT
<213> Mus musculus

<400> 17
Ser Pro Pro Pro Tyr Ser Pro Xaa
1 5

<210> 18
<211> 119
<212> PRT
<213> Homo sapiens

<400> 18
Met Pro Arg Glu Asp Ala His Phe Ile Tyr Gly Tyr Pro Lys Lys Gly
1 5 10 15

His Gly His Ser Tyr Thr Thr Ala Glu Glu Ala Ala Gly Ile Gly Ile
20 25 30

Leu Thr Val Ile Leu Gly Val Leu Leu Leu Ile Gly Cys Trp Tyr Cys
35 40 45

Arg Arg Arg Asn Gly Tyr Arg Ala Leu Met Asp Lys Ser Leu His Val
50 55 60

Gly Thr Gln Cys Ala Leu Thr Arg Arg Cys Pro Gln Glu Gly Phe Asp
65 70 75 80

His Arg Asp Ser Lys Val Ser Leu Gln Glu Lys Asn Cys Glu Pro Val
85 90 95

Val Pro Asn Ala Pro Pro Ala Tyr Glu Lys Leu Ser Ala Glu Gln Ser
100 105 110

Pro Pro Pro Tyr Ser Pro Xaa
115

<210> 19
<211> 1559
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (54)..(407)

<400> 19

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agcagacaga ggactctcat taaggaaggt gtcctgtgcc ctgaccctac aag atg     56
                                                            Met
                                                             1

cca aga gaa gat gct cac ttc atc tat ggt tac ccc aag aag ggg cac   104
Pro Arg Glu Asp Ala His Phe Ile Tyr Gly Tyr Pro Lys Lys Gly His
              5                  10                  15

ggc cac tct tac acc acg gct gaa gag gcc gct ggg atc ggc atc ctg   152
Gly His Ser Tyr Thr Thr Ala Glu Glu Ala Ala Gly Ile Gly Ile Leu
         20                  25                  30

aca gtg atc ctg gga gtc tta ctg ctc atc ggc tgt tgg tat tgt aga   200
Thr Val Ile Leu Gly Val Leu Leu Leu Ile Gly Cys Trp Tyr Cys Arg
     35                  40                  45

aga cca aat gga tac aga gcc ttg atg gat aaa agt ctt cat gtt ggc   248
Arg Pro Asn Gly Tyr Arg Ala Leu Met Asp Lys Ser Leu His Val Gly
 50                  55                  60                  65

act caa tgt gcc tta aca aga aga tgc cca caa gaa ggg ttt gat cat   296
Thr Gln Cys Ala Leu Thr Arg Arg Cys Pro Gln Glu Gly Phe Asp His
                 70                  75                  80

cgg gac agc aaa gtg tct ctt caa gag aaa aac tgt gaa cct gtg gtt   344
Arg Asp Ser Lys Val Ser Leu Gln Glu Lys Asn Cys Glu Pro Val Val
             85                  90                  95

ccc aat gct cca cct gct tat gag aaa ctc tct gca gaa cag tca cca   392
Pro Asn Ala Pro Pro Ala Tyr Glu Lys Leu Ser Ala Glu Gln Ser Pro
        100                 105                 110

cca cct tat tca cct taagagccag cgagacacct gagacatgct gaaattattt   447
Pro Pro Tyr Ser Pro
    115

ctctcacact tttgcttgaa tttaatacag acatctaatg ttctcctttg gaatggtgta 507

ggaaaaatgc aagccatctc taataataag tcagtgttaa aattttagta ggtccgctag 567

cagtactaat catgtgagga aatgatgaga aatattaaat tgggaaaact ccatcaataa 627

atgttgcaat gcatgatact atctgtgcca gaggtaatgt tagtaaatcc atggtgttat 687

tttctgagag acagaattca agtgggtatt ctggggccat ccaatttctc tttacttgaa 747

atttggctaa taacaaacta gtcaggtttt cgaaccttga ccgacatgaa ctgtacacag 807

aattgttcca gtactatgga gtgctcacaa aggatacttt tacaggttaa gacaaagggt 867

tgactggcct atttatctga tcaagaacat gtcagcaatg tctctttgtg ctctaaaatt 927

ctattatact acaataatat attgtaaaga tcctatagct ctttttttt gagatggagt 987

ttcgcttttg ttgcccaggc tggagtgcaa tggcgcgatc ttggctcacc ataacctccg 1047

cctcccaggt tcaagcaatt ctcctgcctt agcctcctga gtagctggga ttacaggcgt 1107

gcgccactat gcctgactaa ttttgtagtt ttagtagaga cggggtttct ccatgttggt 1167
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caggctggtc tcaaactcct gacctcaggt gatctgcccg cctcagcctc ccaaagtgct 1227

ggaattacag gcgtgagcca ccacgcctgg ctggatccta tatcttaggt aagacatata 1287

acgcagtcta attacatttc acttcaaggc tcaatgctat tctaactaat gacaagtatt 1347

ttctactaaa ccagaaattg gtagaaggat ttaaataagt aaaagctact atgtactgcc 1407

ttagtgctga tgcctgtgta ctgccttaaa tgtacctatg gcaatttagc tctcttgggt 1467

tcccaaatcc ctctcacaag aatgtgcaga agaaatcata aaggatcaga gattctgaaa 1527

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa 1559

<210> 20
<211> 118
<212> PRT
<213> Homo sapiens

<400> 20
Met Pro Arg Glu Asp Ala His Phe Ile Tyr Gly Tyr Pro Lys Lys Gly
1 5 10 15

His Gly His Ser Tyr Thr Thr Ala Glu Glu Ala Ala Gly Ile Gly Ile
20 25 30

Leu Thr Val Ile Leu Gly Val Leu Leu Leu Ile Gly Cys Trp Tyr Cys
35 40 45

Arg Arg Pro Asn Gly Tyr Arg Ala Leu Met Asp Lys Ser Leu His Val
50 55 60

Gly Thr Gln Cys Ala Leu Thr Arg Arg Cys Pro Gln Glu Gly Phe Asp
65 70 75 80

His Arg Asp Ser Lys Val Ser Leu Gln Glu Lys Asn Cys Glu Pro Val
85 90 95

Val Pro Asn Ala Pro Pro Ala Tyr Glu Lys Leu Ser Ala Glu Gln Ser
100 105 110

Pro Pro Pro Tyr Ser Pro
115

<210> 21
<211> 517
<212> PRT
<213> Mus musculus

<400> 21
Met Gly Leu Val Gly Trp Gly Leu Leu Leu Gly Cys Leu Gly Cys Gly
1 5 10 15

Ile Leu Leu Arg Ala Arg Ala Gln Phe Pro Arg Val Cys Met Thr Leu
20 25 30

Asp Gly Val Leu Asn Lys Glu Cys Cys Pro Pro Leu Gly Pro Glu Ala
35 40 45

```
Thr Asn Ile Cys Gly Phe Leu Glu Gly Arg Gly Gln Cys Ala Glu Val
     50                  55                  60

Gln Thr Asp Thr Arg Pro Trp Ser Gly Pro Tyr Ile Leu Arg Asn Gln
 65                  70                  75                  80

Asp Asp Arg Glu Gln Trp Pro Arg Lys Phe Phe Asn Arg Thr Cys Lys
                 85                  90                  95

Cys Thr Gly Asn Phe Ala Gly Tyr Asn Cys Gly Gly Cys Lys Phe Gly
            100                 105                 110

Trp Thr Gly Pro Asp Cys Asn Arg Lys Lys Pro Ala Ile Leu Arg Arg
        115                 120                 125

Asn Ile His Ser Leu Thr Ala Gln Glu Arg Glu Gln Phe Leu Gly Ala
    130                 135                 140

Leu Asp Leu Ala Lys Lys Ser Ile His Pro Asp Tyr Val Ile Thr Thr
145                 150                 155                 160

Gln His Trp Leu Gly Leu Leu Gly Pro Asn Gly Thr Gln Pro Gln Ile
                165                 170                 175

Ala Asn Cys Ser Val Tyr Asp Phe Phe Val Trp Leu His Tyr Tyr Ser
            180                 185                 190

Val Arg Asp Thr Leu Leu Gly Pro Gly Arg Pro Tyr Lys Ala Ile Asp
        195                 200                 205

Phe Ser His Gln Gly Pro Ala Phe Val Thr Trp His Arg Tyr His Leu
    210                 215                 220

Leu Trp Leu Glu Arg Glu Leu Gln Arg Leu Thr Gly Asn Glu Ser Phe
225                 230                 235                 240

Ala Leu Pro Tyr Trp Asn Phe Ala Thr Gly Lys Asn Glu Cys Asp Val
                245                 250                 255

Cys Thr Asp Asp Trp Leu Gly Ala Ala Arg Gln Asp Asp Pro Thr Leu
            260                 265                 270

Ile Ser Arg Asn Ser Arg Phe Ser Thr Trp Glu Ile Val Cys Asp Ser
        275                 280                 285

Leu Asp Asp Tyr Asn Arg Arg Val Thr Leu Cys Asn Gly Thr Tyr Glu
    290                 295                 300

Gly Leu Leu Arg Arg Asn Lys Val Gly Arg Asn Asn Glu Lys Leu Pro
305                 310                 315                 320

Thr Leu Lys Asn Val Gln Asp Cys Leu Ser Leu Gln Lys Phe Asp Ser
                325                 330                 335

Pro Pro Phe Phe Gln Asn Ser Thr Phe Ser Phe Arg Asn Ala Leu Glu
            340                 345                 350

Gly Phe Asp Lys Ala Asp Gly Thr Leu Asp Ser Gln Val Met Asn Leu
        355                 360                 365
```

```
His Asn Leu Ala His Ser Phe Leu Asn Gly Thr Asn Ala Leu Pro His
    370                 375                 380

Ser Ala Ala Asn Asp Pro Val Phe Val Val Leu His Ser Phe Thr Asp
385                 390                 395                 400

Ala Ile Phe Asp Glu Trp Leu Lys Arg Asn Asn Pro Ser Thr Asp Ala
                405                 410                 415

Trp Pro Gln Glu Leu Ala Pro Ile Gly His Asn Arg Met Tyr Asn Met
            420                 425                 430

Val Pro Phe Phe Pro Pro Val Thr Asn Glu Glu Leu Phe Leu Thr Ala
        435                 440                 445

Glu Gln Leu Gly Tyr Asn Tyr Ala Val Asp Leu Ser Glu Glu Glu Ala
    450                 455                 460

Pro Val Trp Ser Thr Thr Leu Ser Val Val Ile Gly Ile Leu Gly Ala
465                 470                 475                 480

Phe Val Leu Leu Leu Gly Leu Leu Ala Phe Leu Gln Tyr Arg Arg Leu
                485                 490                 495

Arg Lys Gly Tyr Ala Pro Leu Met Glu Thr Gly Leu Ser Ser Lys Arg
            500                 505                 510

Tyr Thr Glu Glu Ala
        515
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<210> 22
<211> 519
<212> PRT
<213> Homo sapiens

<400> 22

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Met Ser Pro Leu Trp Trp Gly Phe Leu Leu Ser Cys Leu Gly Cys Lys
  1               5                  10                  15

Ile Leu Pro Gly Ala Gln Gly Gln Phe Pro Arg Val Cys Met Thr Val
             20                  25                  30

Asp Ser Leu Val Asn Lys Glu Cys Cys Pro Arg Leu Gly Ala Glu Ser
         35                  40                  45

Ala Asn Val Cys Gly Ser Gln Gln Gly Arg Gly Gln Cys Thr Glu Val
     50                  55                  60

Arg Ala Asp Thr Arg Pro Trp Ser Gly Pro Tyr Ile Leu Arg Asn Gln
 65                  70                  75                  80

Asp Asp Arg Glu Leu Trp Pro Arg Lys Phe Phe His Arg Thr Cys Lys
                 85                  90                  95

Cys Thr Gly Asn Phe Ala Gly Tyr Asn Cys Gly Asp Cys Lys Phe Gly
            100                 105                 110

Trp Thr Gly Pro Asn Cys Glu Arg Lys Lys Pro Pro Val Ile Arg Gln
        115                 120                 125
```

```
Asn Ile His Ser Leu Ser Pro Gln Glu Arg Glu Gln Phe Leu Gly Ala
    130                 135                 140

Leu Asp Leu Ala Lys Lys Arg Val His Pro Asp Tyr Val Ile Thr Thr
145                 150                 155                 160

Gln His Trp Leu Gly Leu Leu Gly Pro Asn Gly Thr Gln Pro Gln Phe
                165                 170                 175

Ala Asn Cys Ser Val Tyr Asp Phe Phe Val Trp Leu His Tyr Tyr Ser
            180                 185                 190

Val Arg Asp Thr Leu Leu Gly Pro Gly Arg Pro Tyr Arg Ala Ile Asp
        195                 200                 205

Phe Ser His Gln Gly Pro Ala Phe Val Thr Trp His Arg Tyr His Leu
    210                 215                 220

Leu Cys Leu Glu Arg Asp Leu Gln Arg Leu Ile Gly Asn Glu Ser Phe
225                 230                 235                 240

Ala Leu Pro Tyr Trp Asn Phe Ala Thr Gly Arg Asn Glu Cys Asp Val
                245                 250                 255

Cys Thr Asp Gln Leu Phe Gly Ala Ala Arg Pro Asp Asp Pro Thr Leu
            260                 265                 270

Ile Ser Arg Asn Ser Arg Phe Ser Ser Trp Glu Thr Val Cys Asp Ser
        275                 280                 285

Leu Asp Asp Tyr Asn His Leu Val Thr Leu Cys Asn Gly Thr Tyr Glu
    290                 295                 300

Gly Leu Leu Arg Arg Asn Gln Met Gly Arg Asn Ser Met Lys Leu Pro
305                 310                 315                 320

Thr Leu Lys Asp Ile Arg Asp Cys Leu Ser Leu Gln Lys Phe Asp Asn
                325                 330                 335

Pro Pro Phe Phe Gln Asn Ser Thr Phe Ser Phe Arg Asn Ala Leu Glu
            340                 345                 350

Gly Phe Asp Lys Ala Asp Gly Thr Leu Asp Ser Gln Val Met Ser Leu
        355                 360                 365

His Asn Leu Val His Ser Phe Leu Asn Gly Thr Asn Ala Leu Pro His
    370                 375                 380

Ser Ala Ala Asn Asp Pro Ile Phe Val Val Leu His Ser Phe Thr Asp
385                 390                 395                 400

Ala Ile Phe Asp Glu Trp Met Lys Arg Phe Asn Pro Pro Ala Asp Ala
                405                 410                 415

Trp Pro Gln Glu Leu Ala Pro Ile Gly His Asn Arg Met Tyr Asn Met
            420                 425                 430

Val Pro Phe Phe Pro Pro Val Thr Asn Glu Glu Leu Phe Leu Thr Ser
        435                 440                 445

Asp Gln Leu Gly Tyr Ser Tyr Ala Ile Asp Leu Pro Val Ser Val Glu
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```
    450                 455                 460

Glu Thr Pro Gly Trp Pro Thr Thr Leu Leu Val Val Met Gly Thr Leu
465                 470                 475                 480

Val Ala Leu Val Gly Leu Phe Val Leu Leu Ala Phe Leu Gln Tyr Arg
                485                 490                 495

Arg Leu Arg Lys Gly Tyr Thr Pro Leu Met Glu Thr His Leu Ser Ser
            500                 505                 510

Lys Arg Tyr Thr Glu Glu Ala
        515
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